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Calcium and Phosphorus in Foods and Nutrition

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BY

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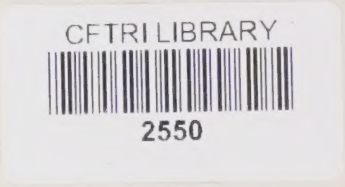

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Preface

MINERAL elements rank with vitamins in their importance to human and animal nutrition, and among the mineral elements calcium and phosphorus are outstanding.

Calcium is, on the one hand, the element needed in largest amount in the building of our skeletal structures, while on the other hand its right concentration in the blood is essential to every heartbeat. Phosphorus, of twin importance with calcium in the bodily framework, enters also into so great a variety of other combinations and functions in the body as to give rise to the saying that, "If the biographies of the elements could be written, that of phosphorus would be the most interesting of all."

For forty years these two factors in our food values and nutritional needs have held a large place in the writer's research and in his discussions with both undergraduate and graduate students. The writing of a manuscript for a more detailed book than this one was interrupted by the Second World War. In the perspective of wartime's intensified studies of national and international problems of nutrition and of food supplies, it was decided that, while all available evidence should be carefully considered, the findings would be of more service if brought into more concise form.

It is hoped that both students and professional workers may find this account readable and applicable to their problems, whether in teaching or in practical dietetics, research, nutrition policy, or food management.

In the introductory chapter enough is explained of the general place of calcium and phosphorus in nature, in agriculture, and in human nutrition to serve as background for the thorough study of the book's five topics. These are: a general account of calcium in the body with especially careful consideration of its forms and functions in the blood and in the bones; a study of the effects of

food and of growth upon the calcium content of the body; a concise but comprehensive summary of the chemical forms and nutritional functions of phosphorus in the body; then the question of quantitative requirements with special consideration of the distinction between minimal-adequate and optimal intakes, the appreciation and understanding of which have been greatly advanced by recent and current researches; a critical examination of foods as sources of calcium and phosphorus and as influencing the behavior of these elements in the body. A final chapter aims both to coordinate the text and to suggest the far-reaching implications of the recent advances which the book aims especially to summarize and interpret. A carefully selected and relatively liberal bibliography serves both as documentation, and to suggest further readings in many directions. For advice on subject matter and for collaboration in the making of the book, special acknowledgment is due to the publisher and to P. C. Jeans, C. S. Lanford, Grace MacLeod, H. H. Mitchell, Ina Padgett, C. S. Pearson, M. S. Ragan, J. M. Schwank, Genevieve Stearns, and the late Dr. T. Wingate Todd; but the author alone is responsible for any errors of interpretation.

H. C. S.

January, 1947

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Calcium and Phosphorus in Foods and Nutrition

Background of the Calcium and Phosphorus Problems of Nutrition

Quantitatively, calcium and phosphorus stand first and second among the mineral elements in our estimates of the ultimate chemical composition of our bodies and those of our domestic animals. We and all other vertebrates owe the form and strength of our skeletal systems chiefly to these two elements.

Calcium and phosphorus are also among the elements of outstanding importance from the viewpoint of the frequency with which one or the other of them is the *limiting factor* determining what happens in nature, in agriculture, or in the development and functioning of our bodies. Clearly, the factors which actually determine what happens, whether in the processes of nature or in those under human control, are the factors of most significance to science. Obviously also, this is true from the viewpoints both of those people seeking purely (as the late Dean Woodbridge put it) to render more intelligible the world in which we live, and those who hold with Francis Bacon that scientific study is also "for the amelioration of man's estate." Quantitatively the position of calcium and phosphorus among the elements which compose our bodies and our environment is shown in Table 1. The first figures for calcium and phosphorus in the left column in Table 1 are those of Mitchell *et al.* (1945).

Oxygen, carbon, and hydrogen are the three most abundant elements of the body; for oxygen and hydrogen make up the water which constitutes about two thirds of the body weight, and these with carbon are contained in all the more abundant organic compounds of organisms. Nitrogen ranks next in quantity in the elementary composition of the body. Also, as the characteristic element of the proteins, nitrogen has, to a large extent, shared the position of

primacy which the name of these compounds has claimed for them for more than a century past. But in our own generation Moore has pointed out that proteins in isolation are inert, nonliving things, and that "what puts life into them is the presence of electrolytes";

TABLE 1

Elementary Compositions of the Body and Its Environment

APPROXIMATE ELEMENTARY COMPOSITION OF THE ADULT HUMAN BODY (ESTIMATED FROM DATA DERIVED FROM VARIOUS SOURCES)		COMPOSITION OF THE EARTH'S CRUST, TO TEN MILES BELOW SEA LEVEL, WITH ITS OCEANS AND ATMOSPHERE (CLARKE)	
Chemical Element	*Percentage*	*Chemical Element*	*Percentage*
Oxygen	65.	Oxygen	50.02
Carbon	18.	Silicon	25.80
Hydrogen	10.	Aluminum	7.30
Nitrogen	3.	Iron	4.18
Calcium	1.596–2.2 [a]	Calcium	3.22
Phosphorus	0.771–1.1 [b]	Sodium	2.36
Potassium	0.35	Potassium	2.28
Sulfur	0.25	Magnesium	2.08
Chlorine	0.15	Hydrogen	0.95
Sodium	0.15	Titanium	0.43
Magnesium	0.05	Chlorine	0.20
Iron	0.004	Carbon	0.18
Manganese	0.0003	Phosphorus	0.11
Copper	0.00015	Sulfur	0.11
Iodine	0.00004	Fluorine	0.10
Cobalt	[c]	Barium	0.08
Zinc	[c]	Manganese	0.08
Others present but of doubtful function		Nitrogen	0.03
		Others in lesser amounts	

[a] Estimates of calcium content vary widely. (See Chapters III and V.)
[b] Percentage of phosphorus varies with that of calcium. (See Chapter IV.)
[c] Believed to be essential, but not yet determined quantitatively.

while Mellor quotes the saying that "if the biographies of the elements could be written, that of phosphorus would be the most interesting of all." Thus we find phosphorus functioning in more than a dozen distinct ways in our bodies (Chapter IV).

While the forms and functions of calcium in organisms have been less studied from the viewpoint of their differentiation, it is well known: (*a*) that but for the essential role of the calcium ions in enabling the blood to coagulate upon occasion, rupture of even a small blood vessel would mean a hemorrhage continuing to a fatal end; and (*b*) that the presence of a right proportion of calcium in the blood is responsible for the alternate contraction and relaxation which constitutes the normal beating of the heart.

The relative insolubility of most of the calcium of our environment is perhaps the chief background fact of the situation in which, with so much calcium in the rocks and soils, there yet is so little in most of our foods.

With phosphorus, the two chief reasons for the occasional existence of a problem of adequacy of nutritional intake are (*a*) that most of the phosphorus in nature is calcium phosphate of the relatively insoluble forms just mentioned in speaking of calcium, and (*b*) that the normal development of the vertebrate body calls for about a tenfold concentration of phosphorus therein as compared with the phosphorus content of our environment (Table 1).

In the general terms of the preceding paragraphs, the background of natural facts is the same whether the calcium and phosphorus problems with which one deals are those of human nutrition or of the feeding of farm animals. We are dealing in the present study with researches of which many were definitely intended to serve both. Primarily, however, this book is written to serve the science and practice of normal human nutrition.

Let us here glance at a few more of the background facts about the plants which we and our animals use as food, the soils in which they grow, and the calcium and phosphorus problems of agriculture and human nutrition.

Plants contain their highest concentrations of calcium in their leaves, while they tend to concentrate their phosphorus in their seeds. Man has evolved largely as a grower of grains, so that the human family now subsists more largely on seeds while cattle live more largely on leaves. To the extent that this happens, it creates a probability of the human family getting relatively more phosphorus than

calcium, while its cattle continue to get relatively more calcium than phosphorus. In so far as present knowledge enables us to take a world view, people in general seem to run more risk of a shortage of calcium, while cattle usually run more risk of a shortage of phosphorus, although when young cattle are fed too largely upon grain they also may suffer from calcium deficiency (Gullickson, Palmer, and Boyce).

The difference just noted does not disappear if the human family is regarded as largely carnivorous. For flesh food augments considerably the dietary supply of phosphorus, while the calcium content of flesh as we eat it is so small as to be negligible from the dietary viewpoint.

Bones are rich in calcium phosphate but most of our people make little dietary use of them. However, if we see a child or a dog gnawing a bone we think of this as supplementing an otherwise low calcium intake; while if we see cattle gnawing bones we think of these cattle as supplementing an otherwise low intake of phosphorus—at least we think so if we have in mind the investigations of osteophagia in cattle briefly noted below.

PHOSPHORUS AS A LIMITING FACTOR

The phosphorus in the earth's crust is rather unevenly distributed, and while it exists essentially as phosphates, these vary from freely soluble salts to extremely insoluble and resistant rocks.

Because of the unevenness of distribution and the differences of resistance to weathering of the phosphates in the rocks from which our soils are formed, the phosphorus contents of soils vary rather widely—from an average of about 0.05 percent, to as high as 0.2 percent in some very fertile soils, and as low as 0.01 percent in the poor soils of the Maryland barrens cited by Hopkins (1910).

As not all of the phosphate in a given soil is sufficiently soluble or sufficiently finely dispersed to be at once available to plants, agricultural chemists have developed certain conventional methods for measuring the supposedly available phosphorus of soils; and data for this latter rather than for the total phosphorus are frequently encountered in agricultural literature.

But even the total phosphorus of soils averages a lower percentage than that of rocks, because the highly resistant *apatites* (igneous phosphate rocks) tend to remain nearly intact in the mountains, while the sandstones and other less resistant but phosphorus-poor rocks disintegrate to form the soils.

Thus agricultural chemistry finds phosphorus to be one of the three elements—nitrogen and potassium being the other two—which are most frequently the limiting factors in crop production, and which, therefore, it is generally good farm practice to apply as fertilizers to the soil. Hence arose the custom of valuing commercial fertilizers according to their contents of nitrogen ("ammonia"), phosphorus ("phosphoric acid"), and potassium ("potash").

Of these three fertilizer elements, phosphorus has been considered as of prime importance by many of the most careful students of American agriculture.

Thus Hopkins concluded that phosphorus is the only element that need be purchased for return to the most common soils of the United States in order to maintain their fertility. He therefore called phosphorus "the key to permanent agriculture" in this country, especially as the maintenance or increase of the phosphorus content of the soil is the prime factor in the growth of clover and other leguminous crops which gather nitrogen from the air and thus enrich the nitrogen supplies at the farmer's disposal.

Harcourt Morgan similarly emphasizes the key position of phosphorus with reference to the permanent fertility of the farms and therefore the feeding of the people. The Agricultural Experiment Station of the University of Tennessee has published a pictorial Popular Bulletin No. 1, entitled *Elements of Life*, which illustrates the importance of phosphorus to our civilization.

The farmer buys phosphate and applies it to his soil in the expectation of larger crops rather than crops of higher phosphorus concentration (specifically richer in phosphorus). Liebig's so-called *Law of the Minimum* taught that whatever element is available in minimal proportion relative to the normal need of a growing plant or animal becomes "the limiting factor" because it was believed that a living organism (and therefore a crop) will grow only to such ex-

tent as is consistent with the specific chemical composition of its kind. Since about 1920, however, several investigators have found, in different parts of the world, considerable areas in which phosphorus-poor soils produce phosphorus-poor forage which in turn results in phosphorus deficiency among the cattle compelled to subsist upon it. (See, for example: Du Toit *et al.*, 1932, 1935; Eckles, Gullickson, and Palmer, 1932; Green, H. H., 1925, 1935; Green, J. R., 1936; Hart, Guilbert, and Goss, 1932; Malan and Du Toit, 1932; Maynard, 1941; Mitchell *et al.*, 1937; Orr and Holm, 1931; Schmidt and Greenberg, 1935; Stewart, 1935; Theiler and Green, 1932; Watkins, 1937; Woodman and Evans, 1930; Bishopp, 1946.) *

Perhaps the first of such reports to command general attention were those of Theiler, Green, and their co-workers, who found that in South Africa there are considerable areas over which the soil is so poor in phosphorus as to induce phosphorus deficiency in the pasture plants and thus in the cattle. Instinct then drives such cattle to the eating of bones (*osteophagia*) which sometimes results in their acquiring fatal infections from decaying carcasses. It was this fact which first stimulated the investigation that finally revealed the phosphorus deficiency. This *aphosphorosis* is more pronounced late in the season when the seeds have fallen from the plants to the ground and are therefore lost to the pasturing cattle.

Using data for "available" phosphorus of soils and for total phosphorus of the pasture grasses growing thereon, Henry and Benjamin (1933) found, in New South Wales, the relationship shown in Table 2.

On the other hand, among animals not entirely dependent upon grazing but partly hand-fed by the farmer, it is doubtless also true, as Forbes has held, that mistakes in feeding too largely upon low-phosphorus parts of the plant may be responsible for much of the ill success which has sometimes been attributed to faults in the soil.

The plan should therefore be also to make full use of available knowledge of the calcium and phosphorus contents of foods and feedingstuffs rather than simply to blame the soil of a region for

* In the preceding paragraph as elsewhere we cite the papers which now seem most serviceable, not necessarily the earliest.

any observed lack of good nutrition among its people or their domestic animals.

The extent of the variations in the calcium and phosphorus con-

TABLE 2

Quantitative Relations of Phosphorus Contents of the Soils and Crops Studied by Henry and Benjamin (1933)

SOURCES	"AVAILABLE" PHOSPHORUS OF SOIL	PHOSPHORUS CONTENT OF PASTURE GRASS
	percent	*percent*
In regions of osteophagia	0.00066	0.119
In non-affected regions	0.00244	0.234

tents of food materials, as ordinarily marketed and consumed, will be taken up in Chapter VI.

CALCIUM DEFICIENCIES IN HUMAN EXPERIENCE

For reasons noted very briefly above and amplified in later chapters, phosphorus deficiency tends to be less common than calcium deficiency in human nutrition, except during infancy when the rapid growth of the muscles creates a relatively high phosphorus requirement and a relatively high incidence of low-phosphorus rickets if the intake of vitamin D is also low. Evidences of calcium deficiency are not usually clear-cut, so probably a great deal of it goes undetected. But the osteomalacia so prevalent in the Orient is now regarded as primarily a calcium-deficiency disease probably often intensified by simultaneous shortage of vitamin D (Hess, 1929; Maxwell, 1934, 1935; Guy, 1936; Aykroyd and Krishnan, 1937). In August, 1946, the Journal of the American Medical Association cited an official report that a high proportion of Australian dietaries show shortage of calcium.

Miles and Feng pointed out that, in those parts of China where osteomalacia is common, a large proportion of the people live mainly on cereal foods with little even of vegetables and almost no milk. And more recently Maynard, upon investigation of the food supplies of Chinese farm families, concluded that they especially needed more calcium. As he considered it economically impracticable for

the majority of Chinese farmers to keep cows at present, he advised an immediate increase in the use of green-leaf foods, while working toward a larger milk supply from either cows or goats in the future.

In south India, where the work of McCarrison and of Aykroyd may be assumed to have improved former nutritional conditions, Aykroyd and Krishnan (1937) nevertheless found calcium deficiencies still frequent even among the people of the educational institutions.

There is much evidence that in the Western World also, calcium deficiencies, while seldom so drastic as to declare themselves unmistakably in the clinic, are frequently present in borderline degree.

Leitch (1937), from an investigation of British patients, reported much of the "arthritis" of middle-aged and elderly people to be a result of long-continued shortage of food calcium; while Ramsay, Thierens, and Magee found that 37 out of 101 women lacked sufficient calcium reserves to maintain normal blood calcium during pregnancy.

Cases have also been described in which lactation resulted in pronounced calcium deficiency, evidently because habitual levels of calcium intake had been low.

Dr. Genevieve Stearns in a personal communication of April 9, 1940, cited the orthopedist at the Iowa University Hospital and Medical School as finding osteoporosis, probably due to low calcium intake, to be very prevalent in the middle-aged people of this country and also of Vienna. (See also the clinical experience of Stare cited in Chapter V.)

Spies finds calcium deficiency one of the several frequent factors in, or complications of, pellagra; and several clinicians in the northern United States consider a shortage of calcium to be a frequent factor in other clinical conditions. Thus many physicians now believe that long-continued low-calcium food supplies and resulting chronic calcium-poor conditions of the body may contribute importantly to chronic illnesses and cases in which people are living on lower levels of health and vitality than they could enjoy if their dietaries or food supplies were better balanced.

Hence the problem of calcium and phosphorus in foods and nutrition now appears to be of great practical importance to human health, especially to the attainment of the higher levels of health and their maintenance through times of special demand. Probably too, calcium, as well as phosphorus, is often a limiting factor in the productivity of farm animals.

In studies of the adequacy of the calcium and phosphorus contents of the food supply to the nutritional needs of the body, the question arises to what extent a suboptimal dietary supply of either or both of these elements can be made good by antirachitic vitamin taken by mouth or produced in the skin by irradiation.

Granting the great importance of the antirachitic substances, and speaking of them (for convenience) simply as vitamin D, to what extent does this vitamin factor enable the body to accomplish the same nutritional results with smaller nutritional intakes of calcium or phosphorus or both?

Vitamin D, given by mouth, or injected subcutaneously, or formed in the skin by the action of light of certain wave lengths, has the property of, in some sense, *mobilizing* calcium and phosphate ions to the advantage of the calcifying cartilage, and thus of the development of the skeletal system. Yet the question remains to what extent this vitamin factor can also *conserve* calcium, or phosphorus, or both, so that the body as a whole is enabled to get along just as well with a smaller nutritional intake of either or both of these elements. And to this question probably no entirely conclusive answer can be given; for one of our highest authorities has recently said: "No one yet knows how vitamin D acts."

Improved intestinal absorption, in those children whose absorption of calcium, or phosphorus, or both, is otherwise poor, is the function of vitamin D which seems to have been most clearly established. It was this which stood out in the critical review presented by Jeans and Stearns to the 1938 meeting of the American Institute of Nutrition. And it is very noticeable that when these investigators are summarizing other evidence, as well as their own, they constantly emphasize the importance of regarding a liberal allowance of vitamin D as something to be provided as a sort of extra insurance in

the interest of good growth and development, and not in any way as an alternative for a liberal calcium intake.

The hypothesis that vitamin D conserves calcium and phosphorus by improving assimilation in those cases in which assimilation of one or both of these elements is otherwise subnormal, and does not so greatly influence the calcium and phosphorus balances of those individuals who have better assimilative powers, now, therefore, has the support of high pediatric authority and apparently goes farther than any other view to reconcile the hitherto conflicting evidence. Also, there is good evidence that vitamin D influences the growth in length of the bones; and it may, in some cases, have other, more local, effects.

Doubtless in some cases nutritional well-being is hampered by simultaneous shortages of both calcium and vitamin D. In such cases vitamin D (or sunshine) may help while also more calcium is needed. Probably, too, there are many cases of simple shortage of calcium.

Calcium in the Body

THE British authorities on food and nutrition, after long and careful study, speak definitely of the "known fact" that a large proportion of family food supplies in England and Scotland have calcium contents too low to be satisfactory. This has also been found, in the United States, through the food consumption studies of Stiebeling and co-workers (1939, 1941); in China, by the survey of Maynard; in tropical America, by Cowgill; in large districts of Australia, of Africa, and of India by other investigators.

There seems no longer room to doubt the reality of a widespread calcium problem in human nutrition. Fortunately, there is also much recent knowledge which can be brought to bear upon the solution of this problem.

DISTRIBUTION BETWEEN SKELETON AND SOFT TISSUES

Of the calcium in the normally developed body, over 99 percent is in the skeletal structures and less than 1 percent is in the soft tissues including the blood and lymph. Thus in their recent analysis of an adult human body, Mitchell *et al.* (1945) found 99.0 percent of the total calcium to be contained in the bones and teeth, while the entire skeletal system including the tendons and ligaments showed 99.47 percent of the total body calcium found.

Analyses of normal adult rats, which had been dissected with special care for this purpose, yielded the following results: In thirty-one females the skeletal system was found to contain 99.32 ± 0.02 * percent, and in thirty-four males, 99.33 ± 0.02 percent, of the total calcium of the body (Bessey, King, Quinn, and Sherman, 1935).

* Throughout this book we use the classical *probable error* as the ± precision measure qualifying any numerical statement of a mean result, or of the difference between the means of two series.

The correct determination of the distribution of calcium in the body was found to necessitate very careful dissecting out of the entire skeletal system, which for this purpose should include, along with the bones, not only the teeth but also the tough or hard tendons and ligaments attached to the bones. When dissection is made as if its object were simply to obtain a clean skeleton for anatomical exhibition, an amount of calcium is removed (with the ligaments and in scraping the muscles from the bones) which may be only a small proportion of the amount belonging to the skeleton but may more than double the calcium content attributed to the muscles or even to the total soft parts of the body. Even larger errors are likely to result when the separation of muscles and bones is effected in part by boiling.

With these facts in mind, one need not be confused by statements, still sometimes encountered, which underestimate the proportion of the total body calcium belonging to the skeletal structures, and overestimate the proportion in the soft tissues. It may be considered as a fact now well-established (at least for the human and rat species) that over 99 percent of the calcium of the normal adult body belongs to the bones and teeth with their attached ligaments and other skeletal structures.

As compared with the above-cited data for normally developed adult rats, smaller percentages of the total body calcium were found in the skeletons of rats which were too young to have completed their normal process of developmental calcification of bone, and in those whose bones were subnormally calcified because of deficiency of calcium in their food supplies. Thus the analyses of two-months-old rats (about half-grown) showed, as the average for eight males, 99.06 percent, and for eight females 99.08 percent of the total body calcium in the skeletal system. And among adult rats whose bodies were calcium-poor because they had grown up on calcium-poor food, careful dissections and analyses of three males and five females showed an average of 98.98 percent of the total body calcium in the skeletal system (Bessey *et al.*, 1935).

In addition to the many other chemical resemblances of the two species, both human babies and baby rats are born calcium-poor as

compared with their parents, not only as to the amount but also as to the percentage of this essential element in their bodies. Hence, in order to accomplish a normal development, the young must increase not only the amounts but also the percentages of calcium in their bodies, and thus have need of relatively higher retentions of calcium than of other body-building materials. Hence, it will readily be seen that the calcium content of the food is important to bodily development, even though the calcium levels in blood and soft tissues may be kept nearly constant by the body's self-regulatory processes. (Blood and lymph are in a very true sense tissues, though we may wish to distinguish them from the tissues of fixed position.)

Other factors may also be important. In his review of the chemistry of calcification, Logan (1940) discusses the endocrine glands, the vitamins, the supply of calcium and phosphate, and the body's acid-base balance as influencing the development of the skeletal system.

STATE OF CALCIUM IN THE SKELETAL SYSTEM

It has long been known that the calcium in the bones and teeth is neither a pure phosphate nor a merely heterogeneous mixture of sparingly soluble calcium salts. The relative amounts of the carbonate and phosphate radicles suggested an approximation to the composition $CaCO_3.3Ca_3(PO_4)_2$.

Bogert and Hastings (1931) showed the similarity of bone mineral from different parts of the skeleton, and Bodansky (1938) emphasized the constancy of bone mineral in different individuals of a species. The x-ray and other studies of recent years have seemed to establish the nature of the bone mineral as a multiple apatite

$$CaX_2.3Ca_3(PO_4)_2$$

in which X_2 represents CO_3, F_2, $(OH)_2$, O, SO_4, or a distribution among these. See, among others, the paper of Gruner, McConnell, and Armstrong (1937) and that of Logan (1940).

The fact of perhaps chief significance from the viewpoint of nutrition is that the calcification of the developing bones and teeth is not a mere precipitation of insoluble calcium salt or salts. Rather,

it is to be regarded as the building into place of tooth and bone mineral in which the relative amounts of calcium and phosphorus can vary but little. While it is logical to bracket together the bones and teeth as parts of the same skeletal system, yet it should also be said that, once formed, the teeth have only a very slow metabolism, even as compared with the bones. Dental literature sometimes presents the appearance of a greater difference of scientific view than actually exists regarding metabolism (exchange of material) in the teeth. Some writers have considered the mineralization of the tooth as so nearly an irreversible process that one should not suggest any possibility of the body taking back anything it had once laid down as tooth mineral. Other students of the subject feel that, while the metabolic mutability of the mineral structures of the bone trabeculae is enormously greater than that of the teeth, yet the difference is one of degree. *Practically* speaking, no doubt, the calcium of the bone trabeculae is available as a nutritional reserve and that of the teeth is not; yet inasmuch as labeled phosphorus atoms can be traced operationally from the circulation into the structure of the fully formed and healthy tooth, a slow exchange of material must occur in the tooth mineral.

The chemistry of calcification has been ably reviewed by Logan (1940) whose discussion is the more effective for being preceded by his careful consideration of the state of calcium in the blood. The ten pages which Logan devoted to this immediate topic should be read in the original (1940, pages 523–533) by those who are interested in the full physico-chemical explanation of calcification.

What is of special interest, from the viewpoint of our present study, is the support given by Logan's theoretical critique of the evidence to the view that the building of bone mineral structures in the skeletal system is something more than a passive precipitation, and that the hard solid form in which the body holds most of the calcium which it possesses is structurally important and yet, at the same time, is more or less available as a reserve upon which the blood can draw to restore a lowered calcium concentration to normal.

Thus our present-day knowledge should correct two oversimplifi-

cations which are still encountered in our scientific literature. It is insufficient to suppose that bones and teeth, once built, are entirely exempt from the general nutritional exchange of material (metabolism, or chemical "turnover") which goes on in the tissues of living bodies. It is also insufficient to suppose that beyond a certain minimum any further calcium which the body may acquire is merely a passive reserve of stored nutriment. For among normally calcified skeletons, those of higher calcium content tend to show a greater development of the bone trabeculae, as illustrated in Figure 1.

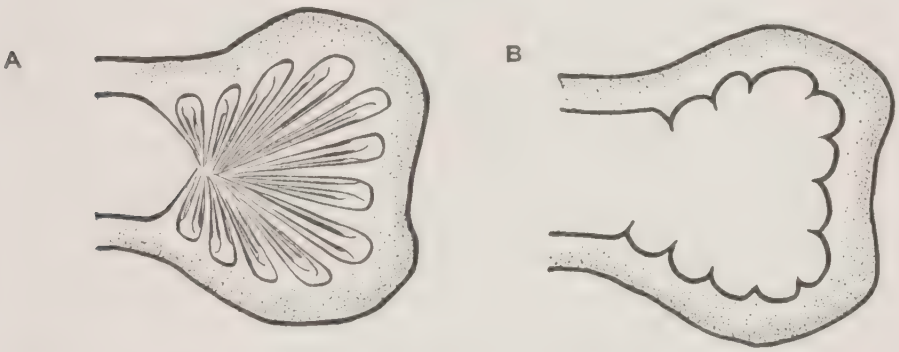

FIGURE 1 Diagrammatic representations of bone trabeculae showing good or poor development according to the level of food calcium intake. A: trabeculae well developed. B: trabeculae poorly developed.

These trabeculae merit special attention.

Whereas the shaft of a typical long bone consists of a more or less cylindrical thick-walled tube of very dense material with a distinct marrow space running lengthwise at its center, the *ends* of such a bone are of a very different description. Here the thick wall is porous and vascular (richly permeated with blood vessels) while into the roughly spherical or cuplike interior cavity at the end of the bone there grow (somewhat like the stalactites and stalagmites in a cave, or like icicles except that they extend from all parts of the surface of this inner cavity toward or to its center) the *bone trabeculae*, also called *spicules* (see Figure 1). These are needlelike structures which, when well developed, tend to meet at the center of the space within the end of the bone, so that they have been likened to the braces in the steel structure of a bridge. This mechanical analogy

seems to have been chiefly in the minds of those who, a generation or more ago, studied these trabeculae and cited them in scientific literature as an example of *functional hypertrophy*. This formal term shows that at that time these trabeculae were regarded as being in one sense hypertrophic deposits of "surplus" material, yet also that (when well developed) these "bracing rods" perform a function in supporting from within the soft porous end of the bone and thus helping to protect it from distortion.

How much one should emphasize this mechanical function may still be a matter of opinion, but there is no room to doubt the importance of the physico-chemical explanation of the functioning of a good trabecular development in helping to maintain or quickly to restore the fully optimal calcium concentration of the blood. In consequence of the vascularity of the ends of the bones, the blood circulates freely through them, and its opportunity to rebuild its calcium content to a fully optimal level is very greatly increased when the trabeculae are well-developed as compared with an otherwise similar condition in which they are but poorly developed. (See diagrams A and B, respectively, of Figure 1.)

A greater than average development of bone trabeculae is therefore a manifestation of storage of what was, in a sense, a surplus of food calcium above the "rock-bottom minimal need," but it is far from being merely a surplus in dead storage—rather it is an ever-valuable addition to the body's working capital.

Thus the so-called surplus of calcium which the body may normally acquire is used constructively. Largely, though not necessarily exclusively, it goes to the strengthening and further perfecting of the skeletal system, through a superior development of bone trabeculae which brings important aid to the maintenance of the normality of the joints, by efficient bracing from within, of the relatively soft ends of the bones. At the same time, this superior development of bone trabeculae functions also as a provision (by virtue of the great surface which the trabeculae present) for the rapid restoration of the calcium level of the blood as it circulates through the vascular ends of the bones.

Thus, even within the normal range, the amount of total calcium

which the body possesses is a variable factor of considerable significance to its nutritional well-being.

CALCIUM CONTENT OF THE BLOOD

Human whole blood contains about 6 milligrams (mg.) of calcium per 100 cubic centimeters (c.c.) or milliliters (ml.). The calcium content of the blood corpuscles is so small as to be only questionably measurable. Blood-calcium studies, therefore, are usually conducted upon the serum (or, less frequently, the plasma) rather than upon whole blood.

The present discussion is chiefly in terms of the calcium-concentration levels found in blood serum.

Overactivity of the parathyroid glands in the secretion of their hormone into the blood (hyperparathyroidism) tends to increase the calcium level and decrease the phosphorus level of the blood serum, and to increase the excretion of both these elements in the urine. This may be of much importance in some cases of disease. In the present book the discussions of calcium and phosphorus assume that the body is possessed of normal parathyroid glands. To attempt adequate discussion of the influence of hyperparathyroidism would lead beyond the scope of this small volume, which is limited essentially to the quantitative distribution of calcium and phosphorus in the body and its foods, and the functioning of these elements in the processes of normal nutrition. Full use of the index and bibliography, however, will help the reader who desires to get into touch with more physiological and medical aspects.

Schmidt and Greenberg (1935) give the normal range of calcium in human blood serum as 9.0 to 11.5 mg. per 100 c.c. or ml. Shohl (1939) estimates the mean value as 10.5 mg. of calcium per 100 c.c. (ml.) of human serum. Bodansky (1938) may be mentioned as among the other modern workers who have reached essentially the same conclusion as to the normal average level of serum calcium in human blood. Mitchell (1939) has given a range of 10 to 12 mg. per-cent * not only for human serum but also for that of cattle, horses,

* For data of the degree of precision of the calcium content of blood serum or plasma, the milligrams per 100 c.c., per 100 ml., or per 100 gm. would all

swine, dogs, and rats, while pointing out that the calcium content of rabbits' blood has been found to be much more variable.

The factors regarded as most important in the maintenance of the normal serum-calcium level are the physico-chemical relationships of the forms of calcium concerned, the availability of a mobilizable calcium reserve, and the functioning of the parathyroid gland.

There is hardly yet a consensus of opinion as to how much the blood-calcium concentration normally changes with age. Denzer, Reiner, and Weiner reported that the concentration of calcium in the blood serum decreases during the first four days of a child's life and then slowly increases to a level somewhat above that of later infancy.

Todd, Chuinard, and Wood found, in blood from the anterior fontanelle of babies: in one series of 630 infants in their first three days of life, an average calcium content of 9.9 milligrams per 100 ml. (with an extreme range of 7.2 to 12.3); and in another series of 573 determinations upon infants four to seven days old, an average of 10.45 milligrams (with an extreme range of 7.5 to 13.9). Blood from the cord, analyzed in 121 of the above cases, averaged 11.27 milligrams (with an extreme range of 7.3 to 16.9). In the last-mentioned investigation a seasonal variation in the serum-calcium level was reported, with slightly higher concentrations in autumn and winter; whereas serum phosphorus was slightly higher in the spring.

The general finding seems to be that the serum-calcium level is slightly higher in infancy than the normal average in adults. Shohl (1939) estimated the average for infants at about 11.2 mg. per 100 c.c. Mull (1936) correlated the variations of calcium concentration in infants with those in maternal blood. The higher serum-calcium level of infants gradually declines and before the age of three years the level is essentially the same as in adults.

The occasionally reported sex differences in human serum-calcium levels are small and have not always been confirmed. It may be considered established, however, that pregnancy, at least in the later months, tends to lower the serum calcium of the maternal blood

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be essentially the same. Hence any or all of these may be covered by the expression "milligrams percent."

(Watchorn and McCance, 1932; Bodansky, 1938; Bodansky and Duff, 1939).

In normally nourished individuals, the intake of food calcium, or even the administration of calcium salts alone, has hardly a measurable direct influence upon the blood calcium level. This fact is doubtless attributable to the efficiency of the bone trabeculae and accompanying "grey sheen" material * in blood-calcium regulation, when the habitual calcium intake has been sufficient for these trabeculae to be well developed (Bauer, Aub, and Albright, 1929, Blunt and Cowan, 1930, pages 31–32; Cannon, 1939; Sherman and Lanford, 1943, pages 125–128).

This calcium reserve is also discussed in connection with the consideration of the state of calcium in the bones (above), and of the problem of optimal calcium intake (Chapter V).

Bodansky and Duff (1941, p. 34) state that in the rat, as in man, fetal development and calcification may be normal under conditions of extreme calcium privation in the mother. This seems to be due to the maintenance of a normal blood calcium in the mother while on calcium-poor food, if she has both "a mobilizable calcium reserve and adequate stimulation of parathyroid activity."

Greenberg and Miller (1941) extended the work of Boelter and Greenberg (1941), who had shown that a marked reduction in the serum-calcium concentration occurred in rats under severe calcium deprivation, into a study of the quantitative changes of serum calcium occurring during the whole course of the calcium deficiency. Notwithstanding considerable daily fluctuations, they considered that the development of a severe calcium deficiency (in rats) led to a progressive decline in the serum-calcium concentration, while the serum-protein concentration remained unchanged, and there were no indications of tetany. These rats had been kept on "stock colony diet" until they reached 94 to 125 grams, then transferred to the experimental diet containing only about 0.01 percent of calcium, on which in the course of thirty-two to sixty-eight days they reached an average maximum weight of 199 grams, after which the body weight

* Described by Todd (1938) on pages 304 to 313 of *Problems of Ageing*, edited by Cowdry (Baltimore: Williams and Wilkins).

declined. From an initial average of 10 to 11 mg. per 100 ml., the serum calcium declined by the end of the first month to an average of 7 to 8 mg., and to 6 to 7 mg. by the end of the second month on the extremely calcium-deficient diet here used. At the end, the body calcium averaged only 0.42 percent as against a normal value (as estimated by the investigators) of 1.0 percent.

STATE OF CALCIUM IN THE BLOOD

While the many investigators of the state of calcium in the blood (more literally its distribution among different states) can not be said to have achieved complete unanimity of opinion, yet there is now essential agreement upon the main facts. For some time it has been recognized that about half the blood calcium exists in such combination with protein as not to be diffusible or ultrafiltrable in the usual understanding of these terms. In different investigations from 40 to 65 percent of the calcium of human blood serum has been found ultrafiltrable depending, at least in part, upon variations in such factors as hydrogen-ion activity (pH), temperature, and type of membrane used, but not on variations in pressure over the range of 50 to 200 pounds per square inch (Watchorn and McCance, 1932; Nicholas, 1932; Miller, M. 1937; Shohl, 1939).

The investigations of McLean and Hastings (1934, 1935 *a*) and of Greenberg and Larson (1935) supported the view that all the serum calcium which is not diffusible or ultrafiltrable may be regarded as combined with serum protein. McLean and Hastings pointed out that the situation in human blood serum may be regarded as an equilibrium between protein, total calcium, and calcium ion; and as all but a trace of the diffusible calcium appears to be ionized, it is hardly necessary to postulate any binding of calcium except with protein. Evidence that the "mass law" adequately describes the relationship between calcium ion and calcium combined with protein is afforded by the experimental work of Chu and Hastings (1938) and of Morison, McLean, and Jackson (1938). Greenwald (1938) and some others, however, do not regard this as entirely conclusive. The evidence is ably reviewed by Logan (1940,

pages 523–527) who also interprets its bearings upon the formation of bone salt.

The outstanding findings of the somewhat extended investigations upon the meaning of diffusibility as applied to calcium in blood serum may be very briefly summarized as in the following paragraphs.

If serum is dialyzed against a very large volume of physiological saline solution * at pH 7.4, the calcium of the serum can be progressively removed by dialysis until no measurable amount remains. However, if the procedure is that known as *compensation dialysis*, where the dialysis fluid contains a mixture of salts alike in amount and kind, to those in serum except for the specific ion under consideration (in this case calcium ion) which is varied until that concentration is determined at which no change in calcium concentration occurs on dialysis against serum, it is found that equilibrium between calcium in the serum and calcium in the dialysis fluid is attained when the calcium concentration in the dialysis fluid is about 60 percent of the total calcium concentration in the serum. It will be evident that the conditions required for perfect compensation dialysis experiments are almost unrealizable, since the amounts and kinds of ions in the particular serum under consideration have seldom been known completely, and if an ionic shift occurs one does not know how this may affect the distribution of calcium ions or the equilibrium between the different forms of calcium in the serum. One should not, therefore, attach too great significance to the exact quantitative values for the "diffusible" calcium fraction as found by this method. It may, however, be stated that compensation dialysis experiments on human blood have indicated that about 60 percent of the serum calcium is normally diffusible in this sense.

A more reliable procedure for measuring this fraction of the serum calcium, but one which, for obvious reasons, has not been applied to man, is the method of vividiffusion. Here the entire blood volume of the living animal is (in the course of its repeated circuits of the

* A solution of sodium chloride of the same concentration as in normal blood serum or plasma.

body) dialyzed against a comparatively small volume of dialysis fluid. Greene and Power (1931), in such vividiffusion experiments, found 50 to 75 percent (on the average, 62 percent) of the serum calcium in the diffusible form.

Another method that has been used to determine the so-called diffusible calcium is ultrafiltration, that is, filtration under pressure through a collodion membrane. Greenberg and his co-workers obtained, by the ultrafiltration method, values for the amount and fraction of diffusible calcium in the blood serum which agreed well with the corresponding data obtained by the vividiffusion technique (Greenberg and Larson, 1935). This is regarded by Schmidt and Greenberg (1935) as a reason for giving precedence to the results obtained by the ultrafiltration technique over those of the so-called compensation dialysis experiments. The reported values by the ultrafiltration method range from 40 to 60 percent for diffusible calcium in human blood, in contrast to values of 60 to 70 percent by the compensation dialysis method. The two methods thus give sufficiently consistent results to leave no doubt that calcium exists in blood serum (and plasma) in two main forms or states, the "diffusible" and the "nondiffusible."

The concept of nondiffusible calcium as bound to protein is now almost universally accepted. Formerly the nature of the association between calcium and protein could not be satisfactorily investigated, because no adequate method for measuring the concentration of calcium ion was at hand. Hence the assumption that all, or practically all, of the diffusible calcium is ionized has only somewhat recently received such experimental support as to command general acceptance.

The current view is that of Hastings and his associates, based on their development of a method for measuring directly the concentration of calcium *ion*. This method depends upon the strength and nature of the contraction of an isolated frog heart, under rigorously controlled conditions such that the calcium ion concentration is the factor limiting the sensitivity of the heart.

Using this method, a study was made of cerebrospinal fluid, as the most easily available approximately protein-free fluid of the

body. The total calcium was determined in each of ten samples of human cerebrospinal fluid, and the calcium ion concentration was estimated by the frog heart method. In practically every case, the total calcium (in the approximately protein-free fluid) was near to, but slightly greater than, the calcium ion concentration. Although the difference, about 6 to 7 percent, is recognized by Hastings and his co-workers to be within the experimental error of the method which they used, they are inclined to the belief that there is a real difference, of the order thus indicated, between the total calcium and the calcium ion concentration, in this practically protein-free body fluid. They cited the finding of citrate in cerebrospinal fluid, and suggested that the amount of citrate may be sufficient to bind an amount of calcium of the order of magnitude of the difference between their findings of total and ionized calcium. McLean and Hastings have also described experiments in which citrate was added to human cerebrospinal fluid and the calcium ion concentration was observed to diminish.

The fact deserving of chief emphasis, however, is that most of the non-ionized calcium of blood serum or plasma is combined with protein; and that this protein-bound calcium is a readily available reserve assisting in the maintenance of the normal calcium ion concentration in the blood.

Those desiring to follow the experimental findings and reasoning in full should read the original papers cited above. The example of experimental data here shown in Table 3 is given as illustrating the liberation of calcium ions from the protein-calcium upon dilution.

It will be seen from the data summarized in Table 3 that with dilution there is a relatively greater diminution of protein-calcium, doubtless because this gives up its calcium to become calcium ions, the ionized calcium falling only to 41 percent, while the protein-calcium falls to 11 percent, of its original concentration.

The excellent review by Logan (1940) is of special interest and value as coordinating the concepts of the state of calcium in the blood and the mineralization of the bones.

The often cited role of calcium in blood coagulation has been reviewed by Howell (1935) and by Ferguson (1936), while more re-

cently Ransmeier and McLean (1938) have investigated further the relationship of calcium ion concentration to the process, and still

TABLE 3

Data of a Typical Experiment by Hastings and Co-workers upon Equilibrium between Calcium and Protein

DILUTION	TOTAL PROTEIN	TOTAL CALCIUM	IONIZED CALCIUM	PROTEIN-CALCIUM
Percent of serum	*mM/kg. H_2O*	*mM/kg. H_2O*	*mM/kg. H_2O*	*mM/kg. H_2O*
100	9.69	3.07	1.35	1.72
75	7.10	2.27	1.15	1.12
50	4.70	1.49	0.90	0.59
25	2.31	0.74	0.55	0.19

more recently Howell (1941) has again reviewed the general theory of blood coagulation from the medical viewpoint and with recognition of the role of vitamin K.

CALCIUM IN THE SOFT TISSUES

According to the data admitted to their compilation by Schmidt and Greenberg (1935) brain averages about 4 to 5, heart about 7 to 8, kidney about 19, liver about 7, muscle about 7 to 8, skin and spleen about 9 to 10 mg. of calcium per 100 g. of the tissue in the wet condition in which it exists in the body. The data of Shohl's (1939) extended compilation are essentially similar. Thus it would appear that the soft tissues as a whole have a level of calcium content not very far different from that of the blood plasma; but generally lower.

Very little seems to be known about the state of calcium in the soft tissues; but there are abundantly striking examples of the fact that the presence of a normal concentration of calcium ion is essential to their proper functioning, including the maintenance of normal permeability of tissue membranes, muscle tone, kidney function, and the activity of the central nervous system (Todd, 1938).

Tetany is, in large measure, believed to be the expression of a low-calcium condition of the body so prevalent as to affect the muscles generally. In the specific case of heart muscle it is well known that

the alternate muscular contraction and relaxation which constitutes the beating of the heart is dependent on the concentration of calcium (ions) in the fluid which bathes it. A Chinese physician, who has had clinical experience in the regions of low-calcium food supply referred to in the preceding chapter, has expressed the clinical opinion that heart ailments are more frequent among people of low-calcium than among those of liberal-calcium food supply.

RICKETS, TETANY, AND OSTEOPOROSIS

The function of this brief section is to summarize, in barest outline, the chemical phenomena of retarded or suspended calcification in the skeletal system which should be depositing bone (and tooth) mineral as a part of its normal development.

The term rickets has been used sometimes in a broader and sometimes in a narrower sense. In the broad sense of Park's modern definition any failure of normal calcification may be called rickets; and as the calcification (or ossification) process is predominantly a deposition of calcium phosphate, its retardation may result from shortage of calcium or of phosphate ions or of both. If the shortage is sufficient to be measurable by chemical analysis of the blood, it may be found to be any one of three conditions: low phosphorus, or low calcium, or a condition in which both calcium and phosphorus are subnormal. The following few words in regard to each of these three conditions are from the viewpoint of the chemical approach.

When the calcium (calcium ion) content of the blood serum is normal but its phosphorus (phosphate ion) content is subnormal, there results the condition of low-phosphorus rickets, sometimes called true rickets because its histology corresponds to Schmorl's "classical" histological descriptions of rickets as he used the term. From the viewpoint of the chemistry of the body as a whole, this is a condition of phosphorus deficiency with concurrent shortage of vitamin D.

When, on the other hand, the shortage of vitamin D is accompanied by plenty of phosphate but a subnormal concentration of calcium (calcium ions) there results a low-calcium rickets often accompanied by tetany.

When there is shortage of both calcium and phosphorus as well as of vitamin D there results "an osteoporosis" which some pathologists have hesitated to call rickets because its histology is different from Schmorl's classical description; but which, from the standpoint of Park's definition, is a third type of rickets.

For full medical discussion, see the monograph of Hess (1929) as mentioned above. Brief descriptions are given by Sherman and Lanford (1943, pages 270–279), and Sherman (1946*a*, pages 447–449).

From the viewpoint of the present volume, this still briefer mention of rickets is included at this point in order to make clear that present usage chiefly follows that of Park which is more simply and comprehensively stated than the older concept which tended to limit the term rickets to what we now call its low-phosphorus type. This situation involves the complication that a statement about rickets may be very ambiguous unless it makes clear whether it refers to the low-phosphorus or to the low-calcium type or to the third type in which there is shortage of both calcium (calcium ions) and phosphorus (phosphate ions). For these three conditions are chemically different, though all are alike nutritionally significant as causing retardation of normal calcification in the developing skeletal system.

CHAPTER III

Effects of Food and Growth upon the Calcium Content of the Body

Passably normal health can be maintained—for a lifetime and even through successive generations—on very different levels of calcium intake and output, and on somewhat different levels of calcium content of body.

Yet differences within the range of this normal zone may influence the degree of positive health and of satisfactoriness of individual and family life history attained; and there is now strong evidence that health and life history may be thus influenced by the level of food-calcium intake even without there having been (constantly) any such difference of blood-calcium level as to be measurable by the methods that have been available. Hence it is important, for the guidance of both human and farm-animal nutrition, to ascertain, by controlled feeding experiments with laboratory animals, the effects of different levels of intake of food calcium upon the composition of the body and the long-time nutritional life history. Recent researches, partly upon people at different stages of life cycle and partly upon animals (of similar nutritional chemistry to the human) which can be experimentally controlled throughout entire lifetimes and successive generations, have thrown interesting new light upon this important problem.

THE TOTAL CALCIUM CONTENT OF THE BODY

According to generally accepted estimates, the human embryo contains at the end of the fourth month of pregnancy but little more than 0.1 gram of calcium; but thereafter the retention proceeds at an accelerating rate so that the body of the baby usually possesses about 20 grams of calcium at birth.

Shohl (1939, p. 50) estimated from the data which he compiled

chiefly from the older literature, that the human body normally averages 0.67 percent of calcium at birth, 1.34 percent at six years, 1.61 percent at ten years, and 1.66 percent at fifteen years and older.

There is general consensus of opinion as to this approximate amount of calcium in the newborn human body; but differences of view exist as to what should be regarded as the normal amount of calcium for the body to contain at ages from six months to one year and onward. Even in relatively recent years, figures for calcium contents of normal adult human bodies have ranged from about 1.6 percent by Mitchell *et al.* (1945) to 3.6 percent by Leitch (1937, 1938).

Leitch's estimate of 3.6 percent of calcium in the normally developed human body assumed that mature bone has 10 percent of moisture, 90 percent of solids, and calcium in fixed proportion to its solids. Shohl, however, gives 46 percent of moisture in mature bone, hence 54 percent of solids or only six tenths as much as Leitch. Reducing Leitch's estimate of calcium in the same proportion gives an estimate (based on the reasoning used by Leitch, but the bone moisture data of Shohl) of 2.2 percent of calcium in the normally developed human body. This is taken as the maximum, and the percentage found in Mitchell's recent analysis as the minimum, of the range given in Table 1 (Chapter I).

If the average baby contains 20 grams of calcium at birth and gains an average of 0.2 gram of calcium a day, it will reach the "chemical maturity" of 1.6 percent of body calcium at a little over three years of age, or that of 2.2 percent of body calcium at an age of five to six years. While the assumptions made for the purpose of this illustration are well within the normal range, it must be emphasized that the evidence shows great flexibility, and particularly that the rate of calcium retention by the body is influenced both by the level of nutritional intake at the time, and by the nutritional history of the individual. Individual variations in the strict sense also seem to constitute a relatively large factor here.

The influence of the food consumed at the time upon the daily retention of calcium by the growing body is illustrated by the data of Dr. Edith Hawley's experiments with the girl E.C., as shown numerically in Table 4 and graphically in Figure 2.

TABLE 4

Influence of Current Intake upon Calcium Retention in a Girl (E.C.)

EXPERIMENTAL PERIOD	CALCIUM IN DAILY FOOD	BODILY RETENTION OF CALCIUM PER DAY
	grams	*grams*
I	0.425	0.222
II	0.748	0.455
III	0.994	0.728
IV	1.273	0.782
V	1.794	0.871
VI	1.262	0.636
VII	1.015	0.544
VIII	0.741	0.403

In this case of a twelve-year-old girl, whose capacity for retention of calcium was evidently good, each of four successive increases of intake induced a further increment of retention; then each of three

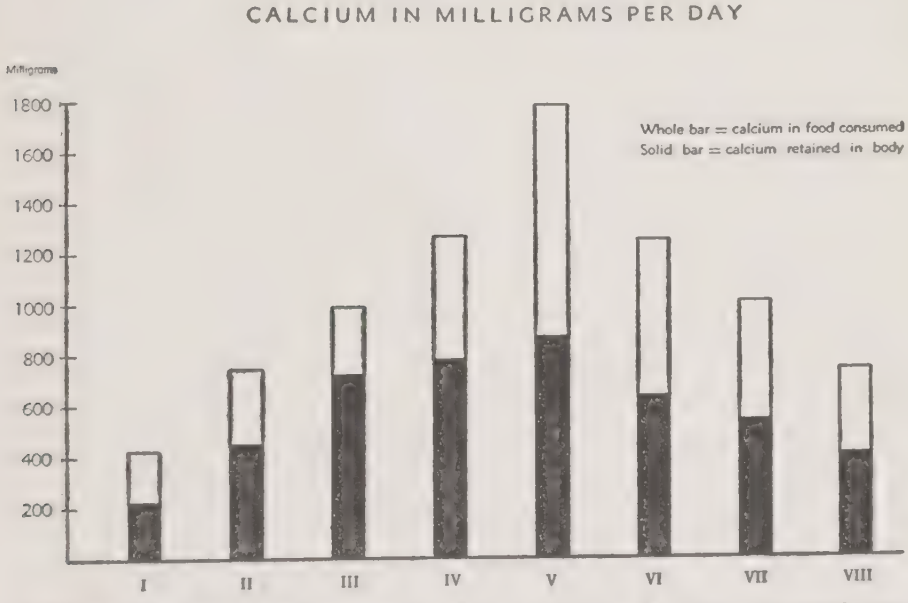


FIGURE 2 Relations between the daily amounts of calcium in food consumed and that retained in the growing human body. The total height of bar represents the daily intake, and that of solid bar the daily retention, in successive periods with the same girl. See text.

successive decreases of intake resulted in a decrease of retention.

Obviously, the higher the rate of retention throughout growth, the higher will be the calcium content of the body at a given age.

Twelve children studied by Sherman and Hawley (1922) showed calcium retentions ranging from 0.147 to 0.622 gram per day while receiving 0.654 to 1.017 grams per day in their food. Each of these figures is an average for nine days of actual balance experiment. Absence of distinct trends within the nine days indicated that the amounts of calcium received during the experiment were fairly similar to what the children had been receiving in their ordinary daily food. The average for each of the twelve children is shown in Table 5.

TABLE 5

Calcium Intake and Retention of Each of Twelve Children (Sherman and Hawley, 1922)

NO.	NAME	AGE	WEIGHT	CALCIUM IN FOOD	CALCIUM RETAINED IN BODY
		yr., mo.	*kgm.*	*gm.*	*gm.*
1.	K.C.	3, 7	16.2	0.885	0.185
2.	C.B.	3, 8	15.2	0.874	0.147
3.	M.O.	4, 7	19.5	0.890	0.183
AVERAGE 1–3			17.0		0.172
4.	A.B.	6	21.4	0.928	0.207
5.	M.P.	6	19.1	0.741	0.230
6.	R.G.	7, 4	25.2	0.894	0.289
7.	R.B.	8, 9	39.3	0.654	0.290
8.	R.T.	9, 6	33.0	0.894	0.273
AVERAGE 4–8			27.6		0.258
9.	L.M.	10, 11	35.4	0.984	0.302
10.	A.F.	11, 5	34.2	0.963	0.310
11.	E.C.	11, 7	30.7	0.964	0.423
12.	L.G.	13, 6	54.9	1.017	0.622
AVERAGE 9–12			38.8		0.414

Averaging the children in three groups we find that those of three to five years of age, averaging 17.0 kgms. in weight, retained an average of 0.172 gram of calcium per day. Those six to ten years old averaged 27.6 kgms. in weight and retained an average of 0.258 gram of calcium a day. Those ten to thirteen years old averaged 38.8 kgm. in weight and retained an average of 0.414 gram of calcium a day. In these children of three to thirteen years calcium retention tended to be proportional to body weight.

Dr. Macy (1942) has published an account of a long-controlled experiment with boys which illustrates strikingly the influence of previous nutritional status upon the retention during a balance experiment. In this case boys who had but ten days of preliminary full feeding stored during a balance experiment 4.6 times more calcium and 7 times more phosphorus than did those who had consumed a liberal diet, including a quart of milk a day, for three months previously.

Here the boys who had had this good feeding for only a short time evidently made up, during the time of the long balance experiment, for previous delays both in skeletal and in soft-tissue growth and development.

As illustrating the influence of calcium intake upon calcium retention in growth, Dr. J. O. Holmes has cited data from Kinsman *et al.* (1939) as follows: With successive intake levels of 333, 555, 704, and 904 mg. of calcium daily, the average daily calcium retention by five girls and six boys of preschool age was 62, 103, 125, and 154 mg., respectively. Here the influence shows itself smoothly but is less pronounced than has often been observed. Perhaps these children had such good stores of body calcium throughout that their bodies had less than average avidity for the larger retentions that many children would have made at the higher levels of intake.

If, from the above-mentioned starting point of 20 grams of calcium in the body at birth, the retention is 0.3 gram of calcium per day, as suggested by McCollum, Orent-Keiles, and Day (1939, p. 183), it would arithmetically be possible for the child to reach, at an age of about seven to eight years, the 3.6 percent of body calcium postulated by Leitch; but Mitchell (1939) and also Shohl (1939)

have definitely expressed the opinion that the body can not be expected to attain so high a calcium content at any age.

Shohl (1939, pages 15 and 16), writing in terms which indicate careful study of the evidence, states that a man's skeleton contains 40 to 60 percent of water, as compared with 70 to 75 percent of water in the muscles, and 15 to 30 percent in the adipose tissues. In his accounting for the water content of the adult human body by distribution in its different organs, he uses Volkmann's figure of 46 percent of water in the adult skeleton, definitely considering Bischoff's estimate of 22 percent and rejecting it as too low. Obviously, then, he cannot concur in Leitch's assumption of the still lower figure of only 10 percent of water in the adult skeleton. The source of the very low figure used by Leitch for moisture in bone (and which is responsible for her unprecedented estimate that the well-developed body should contain as much as 3.6 percent of calcium) is not clear. Bone loses moisture so rapidly upon exposure to air that a report of only 10 percent of moisture in a sample of bone suggests at once that the material as analyzed may have been much drier than it was in the body. It is also conceivable that the material analyzed may have been a sample of the most densely mineralized portion of bone, and that the result therefore is not applicable to the whole weight of the skeleton.

McCollum, Orent-Keiles, and Day (1939, page 184) cite Todd's roentgenological evidence that the skeletons of adolescent American children are commonly less calcified than they should be for their chronological ages, indicating that customary calcium intakes of children should be increased.

At the 1938 meeting of the Society of Biological Chemists, Jeans and Stearns likewise advocated liberal calcium intakes in order to increase the rates of calcium retention and of skeletal development in children. Their data for desirable and practicable retentions at different ages constitute a distinct upward revision of the estimates which most medical writers had previously used.

The hundreds of calcium-balance experiments with children, now on record, afford many illustrations of the fact that different normal children of the same age or size may show quite different rates of cal-

cium retention. This may mean either or both of two things: The greater retention rate may be due to a calcium-deficient condition of body at the beginning of the period of observation of the balance; or the high retention capacity may be a positive characteristic implying rapid advancement toward chemical maturity. Interpretation is hampered by lack of methods for ascertaining the calcium contents of children. Hence the information obtained from experiments with children can be very importantly supplemented by experiments with animals, which can be kept from generation to generation under known nutritional conditions, and with actual analyses of entire individuals for body calcium at different ages.

We now have the records of many hundreds of rats (of known genetic and nutritional backgrounds) which have been thus analyzed at predetermined ages, while their respective litter-mates, grouped in families, have been given otherwise parallel conditions throughout their whole natural lives to determine which of two diets is the better in the long run of a lifetime, or supports better family life histories through successive generations.

Dr. Florence L. MacLeod, then of the Department of Chemistry of Columbia University, studied the effects of diets of differing calcium contents upon the development and body composition, and the changes of body calcium with age and growth, in rats of accurately known backgrounds, kept under strictly parallel conditions (Sherman and MacLeod, 1925).

When twin brothers, of like size and development at the beginning of the experiment, were fed meat and wheat, with and without milk, the difference in skeletal development was as shown in Figure 3. Duplicates of these animals were cremated for analysis and found to contain, respectively, 1.05 percent and 0.52 percent of calcium in their live weights at the same age. The former percentage is normal for the age, while the latter is only half as much. The rat of normally developed skeleton had received about one third of one percent of calcium in the dry matter of his food, while his low-calcium twin had received a diet which contained only about one sixth as much. This drastic shortage of calcium clearly caused a dramatic interference with skeletal development in the first generation.

With less severe shortages of calcium in the food, animals of good genetic and nutritional backgrounds may grow up in apparently normal health and may rear young. But these offspring of low-calcium families are more or less inferior depending on the severity of the shortage of calcium in the family dietary. Thus, in a series in which both branches of the family were fed wheat-and-milk mixtures, in one case milk constituting one third of the food solids while in the other case only one tenth, the result in the second generation was a contrast essentially like that shown in Figure 3.

Toepfer and Sherman (1936) showed, further, that the invisible effects of mild differences of diet upon growth and calcification are more clearly measurable by chemical analysis when the diets to be compared are fed not only to twins but also to cousins whose parents have been reared upon the same respective diets. Testing in this way they found that, on dietaries known to be adequate in other respects, the full advantage to growth and apparent general health and vitality seemed to have been attained at a level of intake of 0.64 percent of calcium in the air-dry food mixture as previously recommended by McCollum and Simmonds, but that further increase to 0.8 percent of calcium may still further increase the rate of calcification. Thirty-day-old rats from families on nearly minimal-adequate diet containing 0.2 percent of calcium in the dry food had about six tenths the percentage of body calcium found in normal adults; those from families whose food contained 0.64 and 0.8 percent calcium had, at the same age, eight tenths and nine tenths, respectively, of the normal adult percentage of body calcium.

As rats do not need to be supplied with vitamin C, the Columbia laboratory has made much use of wheat-and-milk mixtures in feeding experiments in which rats are used for full-life and successive-generation researches upon calcium, protein, vitamin A, and riboflavin in nutrition.

A mixture of five-sixths ground whole wheat and one-sixth dried whole milk (with 2 percent as much table salt as wheat, and with pure water *ad libitum*), which for the convenience of readers we call Diet A *, has been found adequate to the support of normal growth,

* The technical identification of this diet in the records of the Department of Chemistry at Columbia is Laboratory Diet No. 16.

FIGURE 3 Twin brother rats showing effects of diets differing widely in calcium content. See text. (Courtesy of the *Journal of Biological Chemistry*.)

health, reproduction, and lactation, generation after generation. When this was written in January, 1947, rat families in the chemical laboratory of Columbia University were still thriving in the sixty-third generation on this diet. Making the usual allowance of thirty years to a human generation, a comparable dietary test directly upon human beings would need to have been started about the time of Nero and continued ever since without interruption.

Certainly this is an amply rigorous demonstration that this *Diet A* is *adequate*, as the word is commonly used and understood. Yet, while adequate, this diet is not optimal; for our *Diet B* (Laboratory No. 13) is *better*, as shown by hundreds of directly comparable life histories of strictly parallel animals fed through entire lives and successive generations upon each of these diets (Sherman and Campbell, 1924, 1928, 1930).

Of animals reared by Dr. Campbell on these two diets, respectively, large numbers have been analyzed for calcium at different ages (Lanford and Sherman, 1938; Sherman, Campbell, and Lanford, 1939; Lanford, Campbell, and Sherman, 1941) with the results summarized in Figures 4 and 5 and Table 6.

TABLE 6

Calcium Contents at Different Ages in Rats from Diets A and B

AGE	FROM DIET A		FROM DIET B	
	MALES	FEMALES	MALES	FEMALES
	percent	*percent*	*percent*	*percent*
28 days	0.715	0.748	0.734	0.750
60 "	0.677	0.676	0.789	0.901
90 "	0.726	0.830	0.908	1.066
180 "	1.023	1.195	1.032	1.238
1 year	1.090	—	1.096	—

For individuals from both diets it is clear that the *percentage* of calcium in the body (not simply the amount) is higher at the end of growth than either at the end of infancy or in the half-grown individual. Except for those on Diet A during about their second month (where gain in body weight temporarily outruns calcium retention) there is a steady gain with time. Females which have not borne the

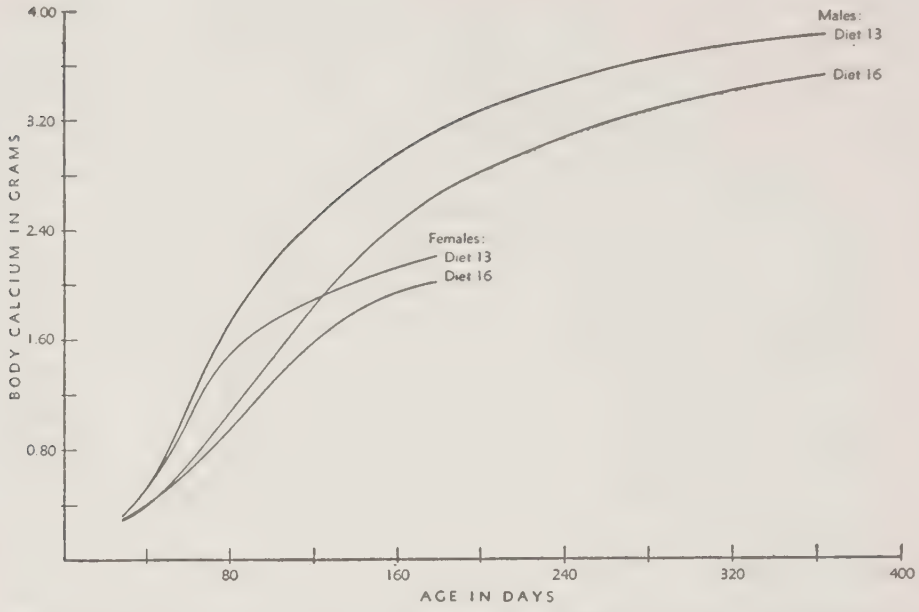


FIGURE 4 Average amounts of calcium at different ages in the bodies of normally nourished rats whose food contained respectively: Diet 13, 0.34 percent, and Diet 16, 0.20 percent, of calcium.

demands of reproduction and lactation contain higher percentages of body calcium than males of the same age and dietary history.

In terms of the natural foods of which they are both composed, Diet B (or 13) differs from Diet A (or 16) simply in containing a higher proportion of milk. In chemical terms, however, the increased proportion of milk means enrichment not only in calcium but also in protein, riboflavin, and vitamin A.

Hence, additional series of otherwise similar experiments have been made to determine the effects of enriching each of these diets in its calcium content only.

Diet 162 was made to differ from Diet A only in containing the same amount of calcium as does Diet B, namely, 0.32–0.35 percent of the air-dry food mixture, or 70 to 80 percent more than in Diet A which contains 0.19–0.2 percent. This enrichment of calcium intake was followed by a more efficient utilization of the food (whether calculated on the basis of its energy value or of its protein content); slightly increased growth; earlier maturity; higher adult vitality, es-

pecially as shown in superior breeding records; a longer period between the attainment of maturity and the onset of senility; and, in less degree, an increase in the average length of adult life, or life expectation of the adult, statistically significant for the males, but not in this case for the females; perhaps because the females on the diet of higher calcium content had borne and suckled more young (Sherman and Campbell, 1935).

In the experiments just cited, the females which received the diet containing the larger amount of calcium reared more young and brought them to a higher average weight at a standard weaning age. When, then, these mothers lived only insignificantly longer than those on Diet A, the question arose whether this was because females are less able than males to invest extra calcium in extra length of life, or because their extra food calcium had been invested in more and superior offspring instead. In females unmated and thus having no offspring in which to invest it, the extra calcium of Diet 162 conferred at least as much improvement in longevity as it did in the cor-

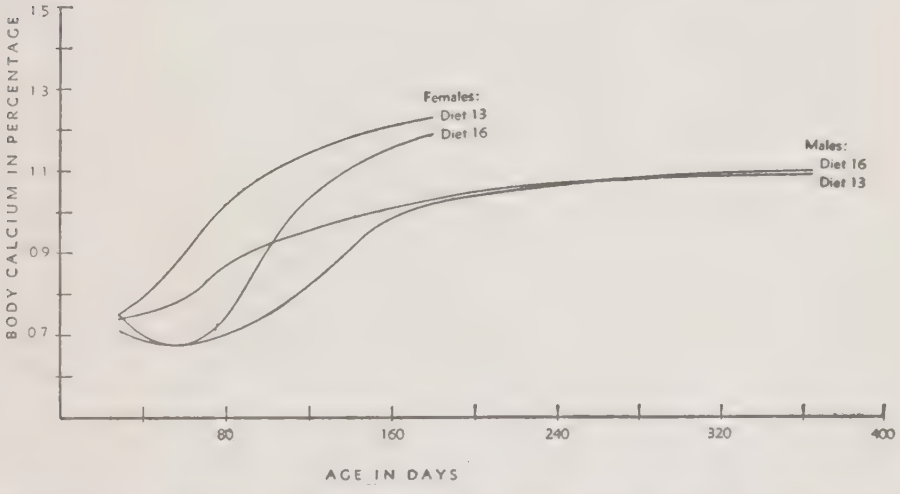


FIGURE 5 Average percentages of calcium at different ages in the bodies of normally nourished rats whose food contained respectively: Diet 13, 0.34 percent, and Diet 16, 0.20 percent, of calcium. Note that for a time corresponding to preschool age in children the lower-calcium dietary supports a lesser-proportionate increase of body calcium than of body weight; but otherwise the growing body consistently increases its percentage as well as its amount of calcium.

responding males (Campbell, Pearson, and Sherman, 1943). Furthermore, it was shown in another series of experiments that with larger increments of dietary calcium the females were enabled both to rear more and sturdier offspring and to enjoy longer lives themselves (Van Duyne, Lanford, Toepfer, and Sherman, 1941).

These last-mentioned experiments were also designed to compare the effects of a threefold with a fourfold increase of the calcium content of Diet A. The comparison was extended to three generations of rats and to a total of 148 males and 209 females essentially equally divided between the two diets containing respectively 0.64 percent and 0.80 percent of calcium in the air-dry food mixture. In most respects the average results were essentially the same on these two levels of calcium intake. However, the females receiving the larger amount of food calcium retained reproductive ability for a longer time, bore and reared more young, and lived longer. It is doubtful whether these differences should be considered as more than accidental, inasmuch as they were small and corresponding differences did not appear with other criteria; but it is perhaps noteworthy that all such average differences as did appear were favorable to the higher calcium intake.

Analyses of rats from the diets containing respectively 0.2, 0.64, and 0.8 percent of calcium showed that the threefold increase of calcium intake was very advantageous in support of normal gains both of the amount and the percentage of calcium in the growing body, and that the further increase from 0.64 to 0.8 percent of calcium in the dry food mixture, while not materially affecting the ultimate calcium content of the body, did expedite the attainment of the adult level of body-calcium content (Lanford and Sherman, 1938).

Similar experimental studies of the effects of calcium enrichment have also been made from the starting point of a basal diet of higher protein, riboflavin, and vitamin A value. This better balanced basal diet was the Diet B mentioned above, which is also the Columbia Laboratory Diet No. 13 and contains 0.32–0.35 percent of calcium in the air-dry food mixture. Enriched to 0.48 percent of calcium, this

becomes Diet 132; and by further enrichment to 0.64 percent of calcium, it becomes Diet 133.

Campbell and Sherman (1945) compared the effects of these three diets when fed through entire natural lives for two generations to parallel families of rats of identical genetic backgrounds. Each family or breeding lot consisted of three females and two males, the sexes being reared together and allowed to breed without restriction. The first generation experimental animals were distributed to their respective diets at the age of twenty-eight days, which may be conventionally considered the end of infancy in the rat. These first generation lots were carefully matched at the beginning, each set of three (to receive Diets 13, 132, and 133, respectively) containing litter-mate males and females, the total initial body weights being the same for each of the three parallel lots. The "second generation" experimental lots were representative offspring of the respective original matched lots, and were started at such times that parallel second generation experimental animals did not differ in date of birth by more than ten days at the most. Thus any seasonal influence was excluded from affecting the comparison of the diets. Twenty-four such families or breeding lots (that is, 72 females and 48 males) were kept throughout their natural lives on each of the three experimental diets which were alike in other respects while containing, respectively, 0.34, 0.48, and 0.64 percent of calcium in the air-dry food mixture. As between the animals on these three diets, the average records showed no significant differences in growth or adult size, rate of development as indicated by the age at which females bore their first young, or in the size of the young at a standard weaning age. But one or both of the diets of higher calcium content gave better results than the diet with 0.34 percent of calcium in the number of young reared, the duration of reproductive life in the females, the length of life both in females and in males, and the percentage of hemoglobin in the blood. Even though these differences taken individually might not appear statistically significant, yet we have here a sufficiently broad and consistent trend to indicate that the nutritional history as a whole is some-

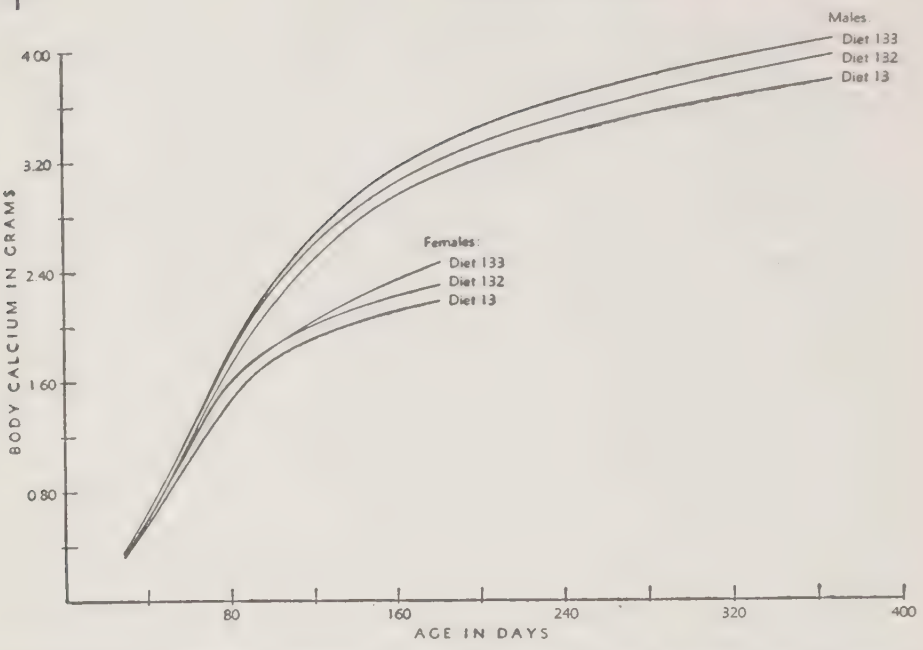


FIGURE 6 Average amounts of body calcium for each sex and as influenced by successive increments of calcium content in otherwise parallel diets of more liberal protein, riboflavin, and vitamin A value than the diets of the experiments of Figures 4 and 5.

what better when the diet contains 0.48 to 0.64, than when it contains 0.34 percent of calcium in the dry food mixture.

The fact that in other experiments with animals of the same colony, but on a less favorable basal diet, a somewhat higher calcium intake appeared necessary for optimal results may, perhaps, have an added interest in relation to the long-standing hypothesis that calcium in addition to its specific functions may also exert a general regulatory or stabilizing effect upon body processes.

Liberal numbers of the offspring of the animals maintained respectively upon Diet B (or 13) and Diets 132 and 133 were analyzed by Dr. C. S. Lanford for the quantitative determination of their body contents of calcium at different ages from infancy to the full maturity of middle life. Figures 6 and 7 show how both the amounts and the percentages of calcium in the body are influenced by increases in the calcium content of a basal diet that initially was already above the level of minimal adequacy both in calcium content and in other respects. Inasmuch as the averages for both sexes and

for all ages studied are readily available to those wishing full detail (Lanford, Campbell, and Sherman, 1941), there are here summarized in Table 7 the mean percentages of calcium in the net fresh body weights of males, with the coefficient of variation of each group and the probable error of each mean.

It will be seen from the data summarized in Table 7 that the coefficients of variation and the probable errors of the means are so small and free from trend that the differences between the mean body-calcium contents found for each diet and age can be used with entire confidence in their significance. They show that the body made better gains in its percentage of calcium when the food contained 0.48 percent of calcium than when it contained 0.34 percent, and that still higher gains were made on a diet containing 0.64 percent of calcium in the dry food.

Thus each of these stepwise increases of about one third in the calcium content of the diet increased the percentage of calcium in the body by about one tenth to one twelfth during the period of most rapid growth, and by about one thirtieth at full maturity.

That the fuller store of body calcium thus obtained was an asset to these animals is highly probable inasmuch as it was accompanied by better levels of blood hemoglobin, better breeding records, and

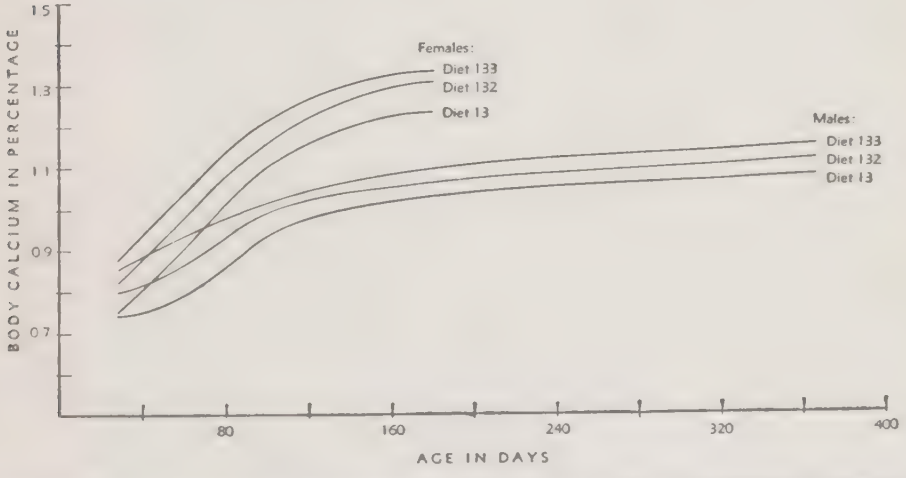


FIGURE 7 Average percentages of body calcium in the experimental animals whose amounts of body calcium are shown in Figure 6. (See text for further explanation of Figures 4 to 7.)

TABLE 7

Calcium Contents of Male Rats from Well-balanced Diets at Three Levels of Calcium Intake; Percentages of Calcium in the Fresh Net Body Weight (Each figure the mean of from 14 to 28 cases)

AGE	FROM DIET 13 WITH 0.34% Ca		FROM DIET 132 WITH 0.48% Ca		FROM DIET 133 WITH 0.64% Ca	
	Mean ± P.E.	C.V.	*Mean ± P.E.*	C.V.	*Mean ± P.E.*	C.V.
28 days	0.734 ± 0.004	3.8	0.799 ± 0.004	4.4	0.854 ± 0.005	4.7
60 "	0.789 ± 0.005	3.8	0.860 ± 0.005	3.2	0.930 ± 0.007	4.3
90 "	0.908 ± 0.007	4.6	0.976 ± 0.009	3.9	1.010 ± 0.009	5.6
180 "	1.032 ± 0.004	2.3	1.065 ± 0.007	4.0	1.093 ± 0.007	3.7
1 year	1.096 ± 0.008	4.6	1.136 ± 0.008	4.3	1.170 ± 0.011	5.9

longer lives, even though there were no significant differences in growth, size, or the ages at which females bore their first young.

Careful expert examination of some hundreds of our animals by the Columbia University department of pathology showed that doubling the calcium contents of our laboratory diets (16 *vs* 162) did not increase the incidence of calcification of arterial walls or of other soft tissues.

The conclusion seems justified that with a diet sufficiently good in other respects the range of optimal calcium intake extended from about 0.34 to at least 0.64 percent of the air-dry food mixture; and that somewhat higher calcium intakes are needed for optimal results if the diet is less favorably constituted in other respects.

The late Dr. T. Wingate Todd examined several rats reared on different diets in our laboratory and considered that without exception the ones from diets richer in calcium were making better skeletal development. His untimely death prevented our carrying this aspect of our investigation farther. Both to him and to Dr. P. C. Jeans the present writer is indebted for cogent expressions of the view that more liberal amounts of calcium in the food, tending to expedite skeletal development in early life and to maintain relatively high body calcium in the adult, may be expected to be advantageous to human beings as well as to laboratory animals.

As bearing upon more detailed interpretations, there is the question as to how the calcium content of the body is affected when growth is either accelerated or retarded by influences other than calcium intake.

The above-mentioned work of Dr. Florence L. MacLeod included (along with thorough studies of the influence of food, age, growth, reproduction, and lactation upon body calcium) a number of analyses of animals whose development had been influenced in other ways. In these cases it was found that animals which had received ample food calcium but were stunted in growth because of other deficiencies in the diet—lack of vitamin A or of thiamine, or too little protein (shortage of amino acid)—had higher calcium

content than normal rats of the same weight, but less calcium than normal animals of the same age.

Some animals were stunted by feeding upon a diet consisting of an originally satisfactory mixture of wheat and milk which had been overdiluted with starch to such an extent that the rats eating *ad libitum* of the diet were barely maintained at constant weight when they would have grown rapidly on the same diet without the starch dilution. This form of dietary stunting also resulted in a calcium content of the body between the amount which would have been normal for the age and the amount which would have been found in a (much younger) normal rat of the same body weight (Sherman and MacLeod, 1925).

In general these analyses showed that the bodies of the young have a fairly strong tendency to increase their percentage of calcium with time even when growth, in the usual sense of increase of body mass, is inhibited—unless, of course, by shortage of calcium itself.

Conner, Kao, and Sherman (1941) found, both with diets of 0.61–0.64 and of 0.77–0.80 percent of calcium in the dry food, that increasing the protein from 14 to 25 percent of the same dry food resulted in an increased rapidity of early growth coincident with an increase in *the amount but not the percentage* of body calcium. In females this percentage showed no consistent change, while in males the more rapid gain in body weight resulted in a retardation of the normal increase in percentage of body calcium. By early middle life all these males had reached the same percentage of body calcium regardless of whether their food had contained 14, 18, or 25 percent of protein. The body calcium content of the females was too much influenced by pregnancy and lactation to permit of similar comparison.

Briwa and Sherman (1941) compared the calcium contents of animals differing in size though of the same age and coming from families on the same diet; that is, animals whose differences were attributable to their individual differences of growth impulse and probably of appetite and not to any difference in the composition of their diets. Different diets were used, not in comparison with each other but in *different series* of comparisons, and in all series

with the same general outcome. This finding was that at the age of twenty-eight days (end of infancy in the rat) the larger animals did not exceed the smaller ones in *percentage* of body calcium. The actual average percentage was slightly lower for the larger animals but at the age then studied the differences in size were not sufficient for a definite diagnosis of negative correlation. When the comparison was carried into the period of most rapid growth where individual differences are larger, Lanford, Campbell, and Sherman (1941) found that in this region the higher body weights (for a given age and composition of diet) were associated with smaller percentages of body calcium.

Also, balance experiments, both with children and with rats, have shown that increased retention of nitrogen (gain of body protein) is not always accompanied by increased retention of calcium (Chen, 1941; Hawks, Bray, Wilde, and Dye, 1942).

Thus at a given age within the period of normal growth, neither larger size as an individual characteristic nor larger size induced by higher protein feeding was associated with correspondingly advanced skeletal development as indicated by the percentage of calcium in the body, or by increased retention in calcium-balance experiments.

Time, on the one hand, and liberality of calcium (and vitamin D) intake, on the other, appear to be the usual determining factors in the development of the body's framework.

The influence of some unfavorable conditions should also be considered.

Brooke and Smith (1933) found loss of calcium from the body to be the outstanding result of their experiments in which rats of an initial weight of 120 grams were fed a diet very poor in inorganic elements. They also noted that tetany frequently developed in these animals, and they conclude that loss of calcium was an important cause of their ultimate death.

Light, Smith, Smith, and Anderson (1934) studied changes due to mineral deficiency by analyses of whole animals which had been fed the diets poor in mineral elements from an initial body weight of 130 grams. When such animals were analyzed after ninety days

of this diet, they showed somewhat less body calcium than the normal controls analyzed at the beginning of the experiment, and much less than the normal control rats of their age at the end of the experiment, that is, at the average age of about two hundred and twenty days.

The findings summarized in the two preceding paragraphs are further clarified by those of Campbell, Bessey, and Sherman (1935) in a series of experiments in which rats were fed from the end of infancy to middle age upon a mixed diet of low calcium content representative of those often encountered in human experience. Here the animals ate *ad libitum* from a homogeneous dried mixture of beef, potato, orange juice, whole milk, whole wheat, white flour, butterfat, lard, and sugar—the whole containing 0.094 percent of calcium in the dry food mixture. These animals grew normally and presented every appearance of good health and development throughout the period of rapid growth between infancy and puberty; but four out of nine females failed to rear any young, whereas in all cases their littermate controls on wheat-and-milk diets of higher calcium content bore and reared young successfully. The second generation on the low-calcium mixed diet just described were about three fourths the average size (for this colony) and grew at about three fourths the average rate. Their coats were rougher, bodies less firm, and they appeared much more nervous than their cousins on the diet of higher calcium content. At one year of age ("early middle age") they exhibited an appearance of incipient senility such as normally develops only at an age of about six hundred days. Among fourteen females of this second generation, at the age of one year, only five had borne young and none had succeeded in rearing any. Analyzed at one year of age both males and females from the diet of 0.094 percent of calcium showed significantly less body calcium than did animals otherwise similar which had received food containing 0.19 percent of calcium in the dry food mixture. Under these conditions the body calcium content was about three fourths to four fifths as much in those whose family food supply was at the 0.094, as in those at the 0.19, percent calcium level.

We saw above that on a given diet those individuals which grow faster in body weight do not necessarily keep pace in their calcium content; and also that an increase in the protein content of the diet tended to increase the body's weight but not its percentage of calcium at a given age within the growing period. Moreover, in additional experiments (work still in progress) in the writer's laboratory, when rats from families on a wheat-and-milk mixture containing 14 percent of protein and 0.19 percent of calcium were given additional protein food, they grew more rapidly and bred at an earlier age, but some (not a majority) of the females broke down in reproduction or before completing the suckling of their young. And here as in the above-cited observations of Brooke and Smith (1933), Light, Smith, Smith, and Anderson (1934), and Campbell, Bessey, and Sherman (1935) there have been nervous symptoms suggestive of tetany and thus of calcium deficiency, and typical fatal cases have shown subnormal amounts of body calcium. There have also been spontaneous recoveries among cases noted as abnormally nervous. One might say in simplest terms that apparently this dietary stimulation of growth and early reproduction may be at some cost of good balance and stability of the nervous system, and that this hazard has usually but not always been successfully outgrown. All these observations are consistent with each other and with the earlier finding of Steenbock, Kent, and Gross (1918) that, with a basal diet of barley, added protein (casein) resulted in improved growth and well-being only when the mineral content of the diet was also improved.

Much that has been confusing in current discussions of, and hitherto recorded findings upon, the effects of different levels of protein consumption may conceivably be explained and reconciled by coordinated long term experiments with systematic simultaneous variations of both protein and calcium (perhaps also vitamin) levels. If it should be found, as so many indications seem now to suggest, that the higher dietary levels of protein are used to better advantage when calcium (and perhaps certain vitamin) intakes are also relatively high, the fact would be important both to fundamental principles of pure science and in its human implications.

Such a relationship might conceivably be (*a*) of a direct chemical nature, or (*b*) more indirect in that it may be not protein specifically but the acceleration of growth which needs increased food calcium for good nutritional balance and stability of the nervous system.

Also, it is *not* to be assumed that calcium is the dominant factor in *all* cases in which good growth is followed by inferior or irregular reproductive performance. Thus Armstrong and Swanson (1943) characterize as "resembling toxemia of pregnancy" a "syndrome of dietary origin" observed in about one third of the animals on one of their experimental diets, although these animals had apparently shown no abnormality of growth.

On the other hand, even an entirely normal performance of reproduction and lactation involves a heavy demand for calcium.

The needs of normal reproduction and lactation. Sherman and MacLeod (1925), Goss and Schmidt (1930), Cox and Imboden (1936*a*), and Boelter and Greenberg (1943) among others, have successively shown the tendency of lactation to deplete the body calcium of the mother rat, while Forbes *et al.* (1922), Hart, Steenbock, *et al.* (1929) among others, have shown the extreme difficulty of preventing large losses of body calcium from heavily milking cows.

The human mother may be relieved of the lactational demand by artificial feeding of the baby; but, as compared with the rat, she has already transferred relatively more of her body calcium to her offspring before its birth.

Hence the demands of reproduction and lactation must both be kept in mind in connection with the fact that the launching of the mammalian offspring puts a special demand upon calcium in one or both of these ways.

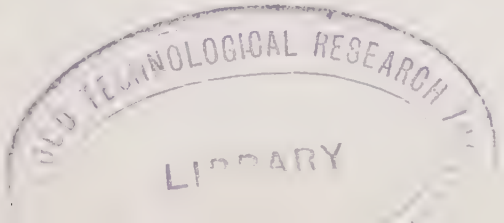
CHAPTER IV

Chemical Forms and Nutritional Functions of Phosphorus in the Body

In the fully developed body the percentage of phosphorus is about half that of calcium. But while over 99 percent of the body's calcium is more or less fixed in its skeletal system, this is true of only about 90 percent of its phosphorus. Thus about five times as much weight of phosphorus as of calcium, and more than six times as many phosphorus atoms as calcium atoms, are found in the body's actively functioning fluids and soft tissues.

Forbes and Keith (1914), in their comprehensive review of the literature up to that date, pointed out that phosphorus compounds enter into practically all physiological functions. Peters and Van Slyke enumerated fourteen ways in which phosphorus compounds function biochemically. Shohl (1939) emphasized the fact that the metabolism of phosphorus is related to that of other mineral elements and to that of proteins, of fats, and of carbohydrates as well. And still more recently Green and Colowick (1944) further point out how varied are the relationships through which inorganic phosphate may be added to or removed from an organic molecule; and organically bound phosphate may be transferred to another part of the same molecule, or to a different molecule.

Among the ways in which phosphorus functions are: as a constituent of the mineral structures of the bones and teeth, as phosphate ions in the fluids and soft tissues, as phospholipids, as phosphoproteins, as nucleoproteins and related substances, as creatine phosphate, as phosphoric acid esters of carbohydrates and their intermediates, as nicotinic acid amide phosphate, as riboflavin enzymes and coenzymes, as diphosphothiamine (cocarboxylase), as pyridoxal phosphate, and in many interrelationships and chain reactions.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AMOUNT AND QUANTITATIVE DISTRIBUTION OF PHOSPHORUS IN THE BODY

Osborne and Mendel (1918) published a brief account, illustrated with graphs of individual cases, of experiments showing that restriction of phosphorus intake inhibits growth, after a rather variable period during which the body to some extent grows up phosphorus poor. Sherman and Pappenheimer (1921*a*, 1921*b*) found that young rats on a diet of white flour plus a phosphate-free salt mixture developed low-phosphorus rickets. Quinn studied quantitatively the influence of age, growth, and food upon the phosphorus content of the body (Sherman and Quinn, 1926; cf. Sherman and MacLeod, 1926). In the extensive series of analyses made by Dr. Quinn, it was found that normal white rats, in the course of their growth and development, multiply by about 150 the amount of body phosphorus with which they are born, while at the same time they multiply their birth weight by about 75. Thus the gain of phosphorus in normally growing up is relatively twice as great as the gain in body-building materials generally. The simultaneous gain of calcium, however, is relatively twice as great as that of phosphorus, because these retentions of calcium and phosphorus during growth are so largely devoted to the normal mineralization of the skeleton, in which the ratio of calcium to phosphorus is about 2:1. The increase in body calcium during growth and development has been discussed in the preceding chapter. Not only in the gains during growth as just noted, but also in comparisons of females with males, and in the effects of the bearing and suckling of young upon the body composition of the females, any extensive trend to gain or loss was in the same direction for the two elements, corresponding to the fact that the bones are the chief storehouse of both calcium and phosphorus and that in the bones these elements exist chiefly in chemical combination with each other. Quinn's experiments and analyses also showed clearly that when the level of calcium intake is suboptimal and the rate of calcium retention is thus retarded, the rate of gain in body phosphorus is thereby retarded also, even though the phosphorus

content of the food is the same (Sherman and Quinn, 1926, pages 674–676).

Whereas the above-mentioned analyses of MacLeod and of Quinn and the subsequent analyses of Lanford (cited in Chapters II and III) give ample information as to the percentages of calcium and phosphorus in normal rats, there is, as we have seen, some difference of opinion as to what we should consider to be the normal calcium content of the adult human body.

The newborn baby has a considerably more mineralized skeleton than has the newborn rat. The human newborn also has a higher percentage of its adult size. We expect the human being, in the course of a normal growth and development, to multiply his birth weight by about 20 to 25, and to increase in greater ratio the amounts of calcium and of phosphorus which the body contained at birth; but in *how much* greater ratio remains uncertain in view of the differences of opinion noted in Chapters II and III as to the normal calcium content of the adult human body. While the discussion in the literature has been in terms of calcium, it follows from what has just been said that higher calcium content means higher phosphorus content also.

For most of both these elements is held in the form of bone mineral and we know that bone mineral does not differ greatly from a calcium: phosphorus ratio of 2:1; and, therefore, that the prevailing difference of opinion as to the normal calcium content and density of mineralization of bone must imply an approximately corresponding degree of doubt as to how much phosphorus we shall consider as normally contained in the fully developed human skeletal system.

Hence, also, the problems of the amount of phosphorus in the body and of its quantitative distribution between the skeleton and the soft tissues are necessarily interdependent. There is no significant change or difference of view as to the phosphorus content of, for example, the muscles or the blood; so if the estimated amount in the skeleton is increased, this increases automatically the estimated percentage of the body's total phosphorus which it holds in its skeleton.

According to the data given by Shohl (1939, page 19) for the adult human body and several of its parts, the skeleton appears to have 87.6 percent, the muscles to have 9.7 percent, and the brain to have 0.8 percent of the total body phosphorus.

If more weight is given to the concept of a more highly mineralized skeleton, Shohl's estimate of the percentage of body phosphorus contained in the skeleton would be increased.

Very probably the blood and soft tissues may contain more kinds of phosphorus compounds than have yet been identified as occurring in them. The longer-known types of phosphorus compounds in blood are grouped by Shohl (1939, p. 27) as phospholipids and acid-soluble phosphorus compounds, and the latter group is subdivided into inorganic, ester, and nucleotide phosphorus. The concentration of inorganic phosphate (phosphate ion) is higher in the blood of infants than of adults.

According to Shohl (1939, p. 185) the concentration of inorganic phosphorus in the blood serum is from 5.0 to 6.6 mg./100 cc. in infancy. This gradually diminishes throughout childhood and becomes relatively stable at 3 to 4 mg. in normal adults.

Ordinarily there is an inverse relation between the concentration of calcium ions and that of phosphate ions in the serum.

Phosphate ion concentration in the blood is temporarily diminished by carbohydrate intake because the metabolism of the carbohydrate is preceded by the formation of carbohydrate phosphoric acid esters, notably glucose-1-phosphate and glucose-6-phosphate.

Mitchell and his co-workers (1945) refer to the skeletal system as containing 90.0 percent of the total phosphorus of the adult human body which was analyzed by them.

In addition to the inorganic phosphates present in the bones and teeth as relatively insoluble mineral crystals and in the blood, lymph, and (other) soft tissues as soluble salts (phosphate ions), phosphorus exists in the body in many organic combinations and these have very important relations to the physical transport and to the chemical transformation of the organic food- and body-stuffs in the nutritional processes. Even while this is being written the work of Cori and others is extending and clarifying our knowledge

of the ways in which the phosphoric acid esters of glucose function in the body's metabolism of the carbohydrate materials which constitute its most abundant sources of energy for the nutritional support of its internal and external activities.

In this chapter we shall review as concisely as possible: first, the effects of phosphorus deficiency, from the viewpoint of the chemistry of nutrition; second, the metabolism of the relatively insoluble phosphates of the bones and teeth; third, the roles of the soluble phosphates (phosphate ions) in the blood and other body fluids; and, finally, glance briefly at those aspects of the functioning of the organic phosphorus compounds of the body which are mostly directly concerned with the nutritional process in the usual sense of the term, as distinguished from general physiology and biochemistry.

EXPERIMENTAL STUDIES OF PHOSPHORUS DEFICIENCY

Because of the possibility that in earlier studies the effects of phosphorus deficiency may have been complicated by vitamin deficiencies not then understood, the present review is confined to relatively recent papers.

Day and McCollum (1939) described their experimental diet as containing only 0.017 percent of phosphorus, which is probably the lowest recorded level of phosphorus content in a dietary otherwise believed to be adequate. Young rats of initial weights of 42 to 54 grams, when restricted to this diet survived from seven to nine weeks, growing slowly during the first five to six weeks of such feeding, and losing weight during the last two to three weeks before death. These animals showed extremely subnormal mineralization of skeleton, and a "progressive disability in walking, standing, and breathing." They were also sensitive to touch, their eyes became watery, and a bloody crust formed on the nose. The difficulty in breathing was attributed by Day and McCollum to the softness of the ribs, which tended to collapse of the thorax. Metabolism-balance studies showed relatively large losses of both phosphorus and calcium from the bodies of these animals. Correlating their x-ray observations with the calcium, phosphorus, and nitrogen bal-

ances as determined for successive weeks, Day and McCollum concluded that the imposition of severe phosphorus deficiency at an age when the growth impulse is normally strong, caused a prompt, marked, and continuous sacrifice of bone phosphate whose calcium was lost to the body while part of its phosphorus was transferred to the growing soft tissues. But later this growth of soft tissue stopped because phosphorus was no longer available in sufficient amounts from the bones.

Their histological studies of these phosphorus-deficient rats showed rickets with marked rarefaction (lack of normal mineralization) of the skeleton. When the paired feeding method was applied so that no more food was available to the adequate-phosphorus animals than the phosphorus-deficient animals ate, no other definite histological differences than those just mentioned as found in the skeleton, could be detected (Follis, Day, and McCollum, 1940).

Jones (1939) appears to have used the diet of next lowest phosphorus content, 0.02 percent, in his studies of phosphorus deficiency as a cause of rickets.

Schneider and Steenbock (1939) fed about twice as much phosphorus and six to twelve times as much vitamin D as in the above-described experiments of Day and McCollum, who suggest (1939, p. 281) that the wide difference in vitamin D supply may be responsible for the apparent difference in the findings of the two investigations as to whether soft tissues have preference over bone in competition for a limited supply of phosphorus.

When Schneider and Steenbock (1939, p. 161) fed their diet containing only 0.04 percent phosphorus to rats of an initial weight of 50 to 60 grams, these animals grew slowly for about four weeks, after which there was loss of weight and in most cases death within six weeks of the beginning of the feeding of this diet. Only one animal out of fifty survived for eight weeks. These animals became rachitic more rapidly than do similar animals on the Steenbock-Black Rachitogenic Ration 2965. "By the 4th week skeletal abnormalities were marked. Muscular tone was poor and a general lethargic condition prevailed. . . . All bones were soft." On

analysis, blood and bone showed marked diminution of phosphorus content; but liver, heart, brain, and muscle did not. Early administration of vitamin D prevented the development of skeletal abnormalities within the time of survival on this phosphorus-deficient diet. In these experiments, the addition of vitamin D to the phosphorus-deficient diet unexpectedly caused a loss of body weight. Schneider and Steenbock (1939, p. 170) conclude that vitamin D induced the utilization of phosphorus by bone, thus depriving the soft tissues of phosphorus with resulting wasting of muscle tissue and loss of weight of the body as a whole.

It is, of course, because of the competition of the rapidly growing soft tissues for the phosphorus brought by the blood that the bones of children of the rickets age so frequently showed low-phosphorus rickets at the same time that calcium was more often than phosphorus the "limiting factor" in the nutrition of the family as a whole. It was the low-phosphorus type of rickets which had been histologically described as true rickets in the literature of pathology and which was therefore the first of the dietetically produced mineral-deficiency conditions to be recognized as an experimental rickets, producible and preventable at will. The diet used in these pioneer experiments consisted of patent flour, calcium lactate, sodium chloride, and ferric citrate; and contained about 0.08 percent of phosphorus in the dry food mixture (Sherman and Pappenheimer, 1921*a*, 1921*b*).

Obviously this diet was deficient in vitamin D and some other factors, as well as in phosphorus, but, as supplementary experiments showed, the test animals of the controlled laboratory colony used had body stores of vitamin D not sufficient to prevent the development of low-phosphorus rickets on this diet but sufficient to permit its prevention by the addition of potassium phosphate to the food. In later diets for the experimental production of low-phosphorus rickets, ground whole grains were used instead of patent flour as the main fuel food with the result that a considerable amount of phytin * was introduced into the rachitogenic diet, and the dis-

* Phytin is the collective name for natural compounds of the hexaphosphoric acid ester of inositol.

cussion of experimental rickets became complicated with the problem of the nutritional availability of phytin phosphorus. It would lead us beyond the scope of this book to enter further into the pathology of rickets, which now commands an imposing medical literature of its own.

However, it should be mentioned that Zucker, Hall, and Young (1941) working from the viewpoint of production of experimental rickets, developed a diet which they characterized as deficient only in phosphate. It contained 0.28 percent of phosphorus and when fed with vitamin D it protected from the pathological anatomy of rickets but did not support a fully normal rate of calcification of the skeleton.

METABOLISM OF PHOSPHORUS IN BONES AND TEETH

As explained in Chapter II, the relatively insoluble salts which give hardness, rigidity, and permanence to the bones and teeth are mineral crystals of the apatite type containing calcium and phosphorus (phosphate ions) in nearly, though not quite, constant proportions, together with a lesser amount of carbonate and usually much smaller proportions of other (substituted) ions.

Thus bone mineral contains about one half as much phosphorus as calcium, and the withdrawal of either of these elements by the blood or lymph from bone means a nearly corresponding loss of the other element from the bone structure.

While the teeth belong to the skeletal system and the bone and tooth mineral are nearly alike, yet histological study of the teeth has led some investigators to regard the enamel at least as being essentially irreversibly segregated from the general metabolism of calcium and phosphorus. Hence (as briefly noted also in Chapter II) we sometimes meet statements to the effect that, once formed, the tooth structure, or at least its enamel, is not any longer concerned in the turnover of materials or subject to resorption under any circumstances; while others speak as though in these respects the difference between the teeth and bones is (in principle) one of degree rather than of kind.

Recent experiments with radioactive phosphorus introduced

into the body as a constituent of a soluble phosphate, such as is normally present in the blood and tissues, throw light upon this question.

Manly and Bale (1939) found that after administration to rats of single doses of disodium phosphate containing radioactive phosphorus (P*), a prompt and rapid deposition of P* occurred in the bones. This was much more marked in the epiphyses, the spongy ends of the bones, with their trabeculae, than in the diaphyses or shafts of the bones. After five days there was evidence of a relative equilibrium between the phosphorus of the blood and that of the bone as a whole. Enough P* was found in the teeth to be taken as evidence that in them, as well as in the bones, there is a measurable phosphorus turnover or metabolic exchange in tooth tissue.

Manly and Bale (1939, p. 125) suggested that quantitative data on radioactive phosphate deposition and turnover should be significant both for fuller understanding of normal nutrition and for medicine. It was by making their observations more quantitative than in earlier work that they were able to show the faster turnover in the ends than in the shafts of the bones and that there is a slow but demonstrable metabolism in the teeth. See especially their Figures 1, 2, and 3 (Manly and Bale, 1939, pages 127, 129, and 130).

Manly, Hodge, and Manly (1940) have suggested that the metabolic turnover of phosphorus in bone may be discussed more conveniently if we make the simplifying assumption that the bone mineral is composed of a labile portion in equilibrium with the blood, and a stable portion which receives phosphorus "in increments."

Logan (1940, p. 52) emphasizes the fact that the normal growth and mineralization of bone is influenced not only by the adequacy of the daily supplies of calcium and phosphate ions but also by endocrine secretions, vitamins, and, in certain species at least, the acid-base balance of the individual.

This, of course, is in entire harmony with Jeans' and Stearns' finding that vitamin D functions (in part, at least) in the intestine

by aiding absorption of calcium or of phosphate where such absorption would otherwise be subnormal. And it is in accord with our general view that such surpluses of calcium and phosphate as are absorbed are likely to be utilized by the skeletal system to support the normal developmental calcification of the essential tooth and bone structures or to produce an increased growth of bone trabeculae which strengthen the ends of the bones and provide a relatively quickly available calcium and phosphate reserve. The more abundant these bone trabeculae, the more promptly is the blood restored to normal composition after any accidental depletion of either its calcium or its phosphate content.

Mitchell, Hamilton, Steggerda, and Bean (1945) while in general referring to the human body they analyzed as if they considered it normal, except for a slight congestion, yet also described (p. 627 of their paper) what appears to have been an incompleteness of skeletal formation and mineralization in the ribs. Their findings for both calcium and phosphorus in this body are therefore given in Table 1 (Chapter I) as the lower limits of a suggested normal range for the calcium and phosphorus contents of the fully developed human body.

SOLUBLE INORGANIC PHOSPHATE

Logan (1940, p. 527) holds that the phosphate of the blood is almost completely ultrafiltrable and diffusible when the concentrations of both calcium and phosphate of the blood plasma are near the normal levels, and that when either is sufficiently increased a colloidal calcium phosphate complex is formed. Normally this is prevented from settling out of the plasma by the "protective action of the protein colloids." He suggested that the available evidence leaves open the question whether or not this form of calcium phosphate differs from that formed in other organic solutions at similar concentrations of calcium and phosphate ions. The state of inorganic phosphate in serum has also been extensively studied and discussed by Schmidt and Greenberg (1935).

Using as a tracer the radioactive active isomer (P*), given in the form of disodium phosphate, Cohn and Greenberg (1938) found

that of the injected or absorbed phosphorus, rats excreted about 20 to 30 percent in the urine within eight hours, while about 3 percent was excreted into the large intestine. The subsequent excretion went on at a steadily decreasing rate. We probably have at present no satisfactory means of translating the time relationship quantitatively to the human metabolism of phosphorus; but, presumably, we might expect in the human body the same trend found in the rat, in that the tissues examined retained the absorbed or injected phosphorus in the following decreasing order: bone, muscle, liver, stomach plus small intestine, blood, kidneys, heart, lungs, and brain. Brain showed a very slow turnover of phosphorus.

It is clear that a very important function of the phosphate ions in the blood is to enter into combination with the organic substances which are undergoing metabolism. Some of these phosphorylations represent a brief functioning of the phosphorus as a constituent of an intermediate compound in the metabolism of the organic substance, while in other cases organic phosphorus compounds formed in the body from organic nutrients and blood phosphate may be built into structural material. The formation of phosphoric acid esters of glucose and some of its metabolic intermediates are typical of phosphorylations from which the phosphorus soon returns to the condition of blood phosphate. On the other hand, the compounds derived in part from the soluble phosphate of the blood serum which become characteristic structural constituents may be illustrated by the nucleic acids of cell nuclei. When, however, a fat or fatty acid is phosphorylated by soluble phosphate of blood, lymph, or cell fluid, the phospholipid formed may function in both ways; part of it as an intermediate in fat metabolism, and part of it as a very important constituent of cell structure and particularly of cell membranes.

Thus the soluble phosphates of the blood, lymph, and tissue fluids, in addition to their function in the buffering and removal of fixed acids, are in dynamic interrelationship, both with the mineral matter of the skeletal system, and with the organic compounds of the soft tissues and the phosphorylized intermediates and catalysts of the active processes of the energy metabolism.

ORGANIC COMPOUNDS AND THEIR FUNCTION

Phospholipids (*Phospholipins, Phosphatids*). The phospholipids, formerly called "phosphorized fats," are important tissue constituents having much to do with the physico-chemical properties of cell membranes and protoplasm. By virtue of their relations to solubility and permeability, they function in the transport of fatty matter in the body. They may also be chemical intermediates in the metabolism of fats (fatty acids), and take part in oxidation-reduction reactions, as well as in some of the immunological reactions which however lie beyond the scope of this book.

Without entering upon the technicalities of molecular structures and classifications, it may here be noted that some of the phospholipids are of added interest to students of nutrition in that they yield choline which functions in some respects like a vitamin.

Phospholipids are produced in both plant and animal organisms. McCollum, Halpin, and Drescher (1912) showed that a hen can produce more phospholipid in her eggs than was contained in the food eaten during the experimental period plus that originally contained in her body; and the ability of the animal body to synthesize phospholipids and other organic compounds of phosphorus has since been abundantly confirmed by growth experiments with animals on rations in which practically all of the phosphorus has been supplied in the form of simple inorganic phosphates.

For recent studies of individual phospholipids see Artom (1945), Gortner (1945*a*, 1945*b*), and Welch (1945); and for the influence of age and diet on the lipid composition of the body see Williams, Galbraith, Kaucher, and Macy (1945).

The promptness with which radioactive phosphorus introduced into the animal body as soluble inorganic phosphate can be found to have passed (in part) into body phospholipid suggests that the synthesis of the latter by the body is a very ready process.

There has been a considerable tendency in the literature of this subject to ask whether phospholipins function as structural constituents of cells and cell membranes or as intermediates in the transport and metabolism of fats or as oxidation-reduction agents.

But these three hypotheses are not mutually exclusive. We may consider one of them as established without letting this fact bias our judgment as to the validity of each of the others. From the fact that cellular tissues retain their lecithin content even in starvation, it may be considered that the lecithins undoubtedly function as structurally essential constituents. There is also strong evidence that they may function as intermediates in the metabolism of fatty acids. See Sinclair (1940*a*, 1940*b*); Chargaff (1942, p. 239–243); Taurog, Entenman, and Chaikoff (1944).

It is to be remembered also that fatty material passes more readily through the covering membrane into the active cell by virtue of the fact that this membrane is itself largely of lipoid nature and by no means so purely a simple protein membrane as casual statements often seem to imply.

Manly, Hodge, and Manly (1940, p. 295) consider, as a result of their studies of the fate of P* after administration as disodium phosphate through stomach tube, that there is rapid establishment of equilibrium between the phosphorus compounds of the blood so that all the fractions of blood phosphorus soon contain essentially the same ratio of radioactive phosphorus to phosphorus.

Hahn and Hevesy (1937), using the radioactive isotope as a tracer to study the formation of yolk lecithin, found that soluble inorganic phosphate, given by subcutaneous injection to a laying hen, is converted by the liver into phospholipids which are carried from the liver to the ovaries by the lymph.

Haven and Bale (1939) found, by intravenous injection of phospholipid containing radioactive phosphorus, that spleen and liver rapidly absorbed the extra phospholipid from the blood, after which the concentrations of the radioactive phosphorus in those tissues decreased slowly while the concentrations increased in the muscles, bones, gastrointestinal tract, and excreta.

Entenman, Changus, Gibbs, and Chaikoff (1940) found that fasting dogs for as long as thirty days did not strikingly change the phospholipid level in the blood, either absolutely or relatively, to other lipids. See also Entenman, Chaikoff, and Friedlander (1946).

Shohl (1939) emphasizes the facts, largely established by the researches of Bloor, that the physiological activity of a tissue tends to correlate with its phospholipid content, and that the latter tends to be retained with great tenacity.

Among more recent general reviews of the phospholipids are those of Hevesy (1940, p. 649–654), Kirk (1940, p. 119–134), Longenecker and Daubert (1945, p. 126–129).

Erickson, Williams, Hummel, and Macy (1937), emphasizing the need for more complete knowledge of the intricate physiological changes that occur in the developing human organism, made unusually complete mineral and lipid analyses of the blood of normal children. They found that phospholipid concentrations (as well as those of other lipid fractions) are in the plasma markedly lower in the preadolescent than in the adult, whereas in the cells the concentration of phospholipid as well as of cholesterol and neutral fat is much more nearly the same for children and adults. Hence they suggested the possibility of using these relationships as indicators of the stage of development during growth. Williams, Erickson, Bernstein, Hummel, and Macy (1937) found subnormal concentrations of phospholipid in the blood plasma of pernicious anemia, and restoration of phospholipid to its normal concentration levels during the course of recovery of the patients after therapy. They also found that the red cells of pernicious anemia in relapse are characterized by subnormal concentrations of phospholipid and, at the same time, by excessive amounts of cholesterol esters. Under therapeutic treatment, cholesterol ester concentration in the red cells diminished and the phospholipid increased, both returning toward normal values.

Ishimoto and Wald (1946) find phospholipids to be involved also in the series of chemical changes upon which vision depends.

For discussion of the theory that phospholipids take part in blood coagulation, see Chargaff (1944, 1945).

Phosphoproteins. The term phosphoproteins is applied to those proteins which contain phosphorus in the form of phosphoric acid

radicles as such and not as nucleic acid or nucleotide. Casein and ovovitellin are the two outstanding members of this group, and their prominence in milk and in eggs, respectively, where they occur alongside considerable amounts of phospholipids, naturally suggests questions as to the nutritional significance of these organic forms of phosphorus in the materials which the body so specifically provides for the conveyance of nutriment. One can hardly, scientifically, conceive otherwise than that there is a nutritional significance in the relative abundance of phosphoproteins and phospholipids in milk and eggs; but indications at present are that the significance lies not in the organic linkage as such, but in the physico-chemical properties of the resulting compounds, and in the fact that they do not directly ionize but yield phosphate ions only as a product of relatively gradual enzymic hydrolyses. Thus they function as reserves of potential phosphate ion and have a value of their own in this connection because their properties and resultant distribution are so different from those of the bone-mineral phosphate. On the phosphate linkage in phosphoproteins, see Lipmann (1933); Sowden and Fischer (1942).

Nucleoproteins, Nucleic Acids, Nucleosides, and Nucleotides; Adenosine Phosphates. The nucleoproteins have still a different distribution from the phosphoproteins; for the former obviously derive their name from their occurrence in the nuclear structures of cells, while the phosphoproteins are found in the colloid dispersions of the special secretions which the higher animals have evolved as a means of safe and efficient transfer of nutriment.

Nucleoproteins are so named because of their occurrence as characteristic components of the nuclei of cells. Both plant and animal cells contain nucleoproteins—proteins holding nucleic acid in chemical combination. The nature of the nucleic acid may vary with the plant or animal or even with the organ in which it is found; for example, triticonucleic acid from wheat, thymonucleic acid from the thymus gland.

Shohl states that the nucleic acid phosphorus constitutes about

one half of the total phosphorus in those organs which are rich in highly nucleated cells: for example, in the thymus, the pancreas, and the spleen.

In the nucleic acids the phosphoric acid radicles are combined with purines, pyrimidines, and carbohydrates, as nucleotides and nucleosides.

Recent research emphasizes the relationships of the phosphate radicles of the nucleoproteins with those that function in carbohydrate metabolism and muscular work, especially through adenosine-phosphate, -diphosphate, and -triphosphate.

While the pyrimidine part of the nucleic acids—notably its adenosine or adenylic acid—thus enters into several important nutritional interrelationships in the body, the purine parts of these same nucleoproteins and nucleic acids are partially oxidized to yield uric acid which is excreted from the body either as such or as its salts, the urates.

The nucleic acids are therefore of great biochemical interest, on the one hand, because of their relations to all the important functions of the nucleoproteins in cell activities and bodily reproduction; and, on the other hand, because of their relations to the nucleotides which are being found so important to the enzyme-coenzyme systems of intermediary metabolism and tissue respiration. Here the hydrolysis-products of nucleic acid and the phosphorylation products of at least three of the vitamins (thiamine, riboflavin, and nicotinic acid or its amide) merge in the complex picture of the functioning of the B vitamins, the metabolism of the carbohydrates (including that of their intermediates), and the chemistry of muscular work and tissue respiration. These and some interrelated factors are briefly mentioned in the paragraphs which follow. For fuller discussions see the reviews by Elvehjem *et al.* (1939); Allen (1941); Green and Colowick (1944); Loring (1944); Gulland, Barker, and Jordan (1945). See also the papers of Kalckar (1944); Kalckar, Dehlinger, and Mehler (1944); and Lehninger (1944), Gulland, Barker, and Jordan (1945).

Kalckar (1945*a*) furnishes evidence that when purine nucleosidases split nucleosides, the 1-phosphoribose rather than free ribose

is liberated; and he suggests that the enzyme catalyzing this equilibrium reaction be called nucleoside phosphorylase in analogy with Cori's polysaccharide phosphorylase and Doudoroff's disaccharide phosphorylase.

The work of Lehninger (1945) brings out the relation of adenosine polyphosphates to the oxidation of fatty acids, probably through the formation of fatty acid acyl phosphates as intermediates.

For recent work, and still other aspects of the chemical natures and nutritional functions and significance of this group of substances, see Kalckar (1944); Kalckar, Dehlinger, and Mehler (1944); Lehninger (1944); Rapoport (1945); Rapoport and Nelson (1945); Schneider (1945); Singher and Meister (1945); Polis and Meyerhof (1946); Zittle (1946*a*).

Creatine Phosphate (*Phosphocreatine, Phosphagen*). The part played by creatine phosphate or phosphocreatine, sometimes called phosphagen, in carbohydrate metabolism and muscular activity had been extensively investigated before the discovery of the functioning of the vitamin-phosphoric acid compounds in these and the related processes (Eggleton, P., 1929; Fiske and SubbaRow, 1929; Cori and Cori, 1933–1935; Eggleton, M. G., 1934; Lohmann, 1938).

Among the discussions taking account of both lines of advance are those of Barron (1941) and of Cori and Cori (1943, 1945). Detailed discussion of the important relations of creatine phosphate to muscular work and carbohydrate metabolism would lead us beyond the scope of this book.

Phosphoric Acid Esters of Carbohydrates and Their Intermediates. Bodansky (1938, p. 323–330) gives a good account of this subject up to 1938. The view that phosphorylation plays an important part in the metabolic transformations of carbohydrates and their intermediates is now well established, though there are still rival theories as to the chemical mechanisms involved. According to Cori and co-workers, the first step in the utilization of glucose by animal tissues, a step common to its transformation to glycogen and its oxidation is (catalyzed by hexokinase): glucose + adenosine tri-

phosphate → glucose-6-phosphate + adenosine diphosphate (Cori 1941; Price, Cori, and Colowick, 1945). See also Cori and Cori (1941, p. 151–156); Sowden and Fischer (1942); Evans (1944, p. 187–191); Green and Colowick (1944); Kaplan and Greenberg (1944); Meyerhof and Beck (1944); and C. F. Cori's Harvey Society Lecture of 1946.

The experiments of the Coris (1945) and of Colowick and Price (1945*a*) have clarified further the central position of glucose-6-phosphate.

Kleiber (1945) quotes Soskin's remark, based largely on the reviews of Baumann and Stare (1939) and of Kalckar (1941), that the "transfer of phosphate groups" is the primary type of chemical reaction through which active cells derive "useful energy" from the organic foodstuffs. See also Cori, Stein, and Cori (1945); Drabkin and Meyerhof (1945); Lardy and Ziegler (1945); Utter, Wood, and Reiner (1945).

Nicotinic Acid Amide Phosphates; Pyridine Nucleotides. The functioning of the pyridine nucleotides and related compounds is discussed in relation to oxidation-reduction by Barron (1941, p. 6–10).

While we may be only at the beginning of an understanding of these compounds, they already seem to be importantly related to the phosphorus-containing nutritional factors mentioned both in the paragraphs just preceding and in those which follow. Here, again, it seems that the reader will be best served by selected references in the bibliography and by a renewal of the recommendation that he examine the literature for papers which may have appeared since this was written.

Riboflavin-Phosphate-Protein Enzymes and Coenzymes. Riboflavin functions in the body largely in combination with phosphoric acid, and through phosphoric acid with protein. The tissue respiratory enzymes, starting with "Warburg's yellow enzyme" and growing rapidly to a group of seven or more, are largely of this character.

They seem to function importantly (*a*) in the metabolism of fuel for the energy transformations essential to the work of the body

as a whole and (*b*) in the maintenance of such condition and tone of the tissues as constitutes an important part of the body's means of preserving its health and resilient vitality.

Interesting interrelations between the riboflavin and the thiamine enzymes and coenzymes are appearing also in the vitamin literature.

Diphosphothiamine (*Thiamine Pyrophosphate, Cocarboxylase*). It is now well established that thiamine functions in nutrition at least largely in the form of the phosphorylation compound which has been variously called diphosphothiamine, thiamine pyrophosphate, and cocarboxylase. This assists in carrying the carbohydrate metabolism through the pyruvic acid and lactic acid stages. Barron (1941, p. 13) considers that there is also adequate evidence that the phosphorylation of thiamine is brought about by phosphoric acid transport from adenosinetriphosphate.

There are also interrelations between the thiamine-phosphate and riboflavin-phosphate factors—and perhaps also the nicotinic acid compounds—in the functioning of the complex enzyme-coenzyme system having to do with tissue respiration and carbohydrate metabolism (Lohmann, 1938, p. 132–133; Elvehjem *et al.*, 1939; Sowden and Fischer, 1942; Ellis, Zmachinsky, and Sherman, 1943).

Pyridoxal Phosphate (*Codecarboxylase*). This phosphate of vitamin B_6 or pyridoxine has been shown by Lichstein, Gunsaulus, and Umbreit (1945) to function as coenzyme both of amino acid decarboxylases and of glutamate-aspartate transaminase.

Linked Reactions in Oxidation-Reduction Tissue Respiration, and Carbohydrate Metabolism. The literatures of these related fields abound in postulations of linked reactions. As a single example, Barron (1941, p. 23) has emphasized the dual view of participation of phosphorus compounds in oxidation-reduction systems and the participation of enzymic reversible systems in phosphorylation processes.

Phosphatases and Local Factors. Phosphatase signifies an enzyme which hydrolyzes a phosphoric acid ester, thus liberating phosphate ion. Robison (1932) proposed the theory that the mineralization of the normally developing bone depends, in part at least, upon the local increase of phosphate-ion concentration at the site of mineralization by the action of phosphatase upon phosphoric acid esters brought by the blood.

There seem to have been two main lines of criticism of this theory. (1) It is held by some that the phosphoric acid ester content of the blood is not sufficient to permit of a very marked increase of phosphate ion concentration through increased phosphatase activity. In reply to this it may be said that, however low the concentration of phosphoric acid ester in the blood, this is to be regarded as in equilibrium with the substances from which it is formed, so that, as its phosphate is liberated and removed from solution by transformation into bone mineral, the ester would presumably be quickly replaced and thus would become a means of active supply of phosphate even though the phosphoric acid ester content of the blood might not be high at any one time. (2) Phosphatase activity has been found to be demonstrable with so wide a variety of tissues and fluids that it cannot be regarded as characteristic of bone, or of the cartilage which is being transformed into bone by mineralization. Nevertheless it is entirely conceivable that there may be "a local factor" at the site of mineralization which catalyzes the activity of phosphatase there.

McLean reported in a lecture in 1941 that in collaboration with Lipton, Barron, and Bloom he had taken up an active investigation of the nature of the local factor specifically concerned with the mineralization of the developing bone.

While this bears most obviously upon the prevention of rickets, it may also have important bearings upon the very complicated problem of the interrelationships of the different kinds of phosphorus compounds concerned in the body's varied nutritional processes. (Green and Colowick, 1944; Kalckar, 1945*a*, 1945*b*; Sendroy, 1945.)

Interrelations and Outlook. Much thought has been given to the endeavor to arrange the topics of the foregoing pages in such manner as will make for convenience and ease of transitions in reading. Yet there is no way of presenting the whole in a simply straight chain sequence of steps, because the actual relationships of the facts in this field are too complex. In handling wartime problems the National Research Council, for the sake of speed both in the formulation and in the use of its Recommended Dietary Allowances, made the simplifying assumption that dietaries and food supplies adequate in other respects may be trusted to contain satisfactory amounts of phosphorus. Doubtless this is true if we think only of minimum needs, but with phosphorus playing such vital and far-reaching parts in nutritional science as it now does, we shall doubtless continue to give it high regard in our permanent considerations of optimal nutrition and of the food values through which the needs of such nutrition are to be supplied.

CHAPTER V

Calcium and Phosphorus Requirements: The Problem of Necessary and Optimal Intakes

THE fact that the human body, like that of many other species, is born calcium poor and so needs relatively more of calcium than of other body-building elements in its food, at least throughout a long period of growth and development, has led to many quantitative studies of the calcium requirements of nutrition.

These studies have been of two main types: calcium-balance experiments with human subjects of different ages, and experimentation with animals of suitably chosen species to determine the effects of different food-calcium levels upon the composition of the body and the nutritional life history.

Careful comparative study of the findings of both types of experimentation has now shown that the minimal food-calcium intake which appears adequate in experiments covering short segments of the life cycle must be considerably increased for *best* results in the long run of entire lifetimes and successive generations.

Unfortunately the improvement of nutritional well-being which should result from this new knowledge is being retarded by the slowness of appreciation of the important bearings of the animal experimentation upon the human problem. The role of calcium is so closely parallel in the nutrition of the human and the rat species that the general relation between level of intake and long-run nutritional well-being established by full-life and successive-generation experiments with rats doubtless holds essentially true for people, even though we are not able to set a precise quantitative relationship between the amounts needed by the human and the rat species respectively.

The broad problem of calcium requirement in human nutrition has therefore three main aspects. First, because logically simplest and most objective, is the requirement for normal maintenance in human adults. Second, the allowances to be made for the obvious additional needs of pregnancy, lactation, and the growth and development of the young. Third, the additional overall allowance or attitude, indicated by the principle taught by McCollum and recently supported and extended by very comprehensive research upon long-run effects of different calcium levels, that there is or may be an important difference between the minimal adequate and the optimal in nutrition.

MAINTENANCE REQUIREMENT AS JUDGED BY CALCIUM-BALANCE EXPERIMENTS

In the study of requirement by the balance method, the general plan is to find by experiment what minimum amount of calcium must be supplied to enable the body to establish and maintain an equilibrium between its intake and its output of calcium.

In experiments made exclusively for this purpose, the ideal plan is to start with a uniform daily diet good in all other respects but containing only about as much calcium as will probably meet the minimal need for the maintenance of equality between the total bodily intake and the total bodily output of calcium. Among well-nourished people it is obviously possible that previous dietary habit may have involved a larger daily intake than the minimum which the experimenter seeks to ascertain. The determination of the balance * should therefore be continued in a sufficient number of successive periods of several days each to give the body opportunity for adjustment. The degree of regularity of response from day to day will determine how many days long each balance period should be, and how many balance periods there should be in a continuous series. Or, as it may also be expressed, the continuous series of experiments should be long enough to enable the body to show its

* As in bookkeeping, a *balance* is struck whenever intake and output are accurately measured and compared. It is only when such measurement shows intake and output to be *equal* that the word *equilibrium* is used.

true response free from the "lag" effect of its previous food-and-nutrition habit. When the true balance has thus been ascertained, if it is found that the body's output was less than or just equal to its intake, the intake level should be lowered and the determination of balance repeated. If, on the other hand, the output, after sufficient opportunity for adjustment, remains higher than the intake level, then the latter should in a series of stepwise experiments be, little by little, increased until the minimal level that permits equilibrium is found. This minimum equilibrium level should, for a given subject, be the same when approached from above and when approached from below. Experiments meeting all these conditions and fully controlled in all respects, tend to become not only very exacting but also almost prohibitively time-consuming and expensive. Possibly at some future time the means will be provided for making such thoroughly planned and controlled long-time series of calcium-balance experiments with larger numbers of human subjects. But at present, we are called upon to use the available data as best these data can be used in the guidance of human nutrition.

Upon critical examination of the laboratory data of the balance-experiment type in which calcium intakes and outputs of human adults have been determined by the quantitative methods of chemical analysis, we find, as Steggerda and Mitchell (1946) emphasize, very considerable physiological variations among healthy people. Hence, in order that an average result shall be broadly applicable, it should be the average of as many normal human adults as practicable.

Hence it is undoubtedly better to use the data of more balance experiments than simply the few that have been made for this exclusive purpose and made in the ideal stepwise way leading to precise equilibrium. To confine ourselves to such ideal cases would admit to our general average the data of too few subjects to be regarded as an adequate sample of the human adult population. Clearly there is need of some method of critical sifting with statistical or graphical interpretation, or correction, of the data of other cases in which the experimental and analytical work were pre-

sumably accurate but intake and output were not necessarily brought into exact equilibrium. Three general types of such procedure are recognizable and can be illustrated by specific references.

The earliest comprehensive study of the then available data involved critical examination of original accounts of balance experiments individually, and the tentative use of the data of the body's output as an indication of its need in those cases in which a relatively small negative balance was found under adequately controlled conditions. It was recognized that this simplifying assumption would tend to low results; and for this and other reasons it was recommended that the tentative minimum average of 0.45 gram per man per day found by this method, be increased 50 percent, that is, to 0.68 gram per man per day, as a tentative guide to dietary allowances, or so-called dietary standard (Sherman, 1920*b*).

Leitch (1937, 1938) compiled data of intake and of corresponding bodily gain or loss of calcium in many of the same early experiments just mentioned, and also of others which she compiled from the literature of the intervening seventeen years, and estimated, by a graphical method, the point at which the numbers of plus and minus balances would theoretically be equal. Maintenance requirement, in this sense, she placed at 0.55 gram per day for normal men and women.

Outhouse and her co-workers (1941) and Steggerda and Mitchell (1941) working at the University of Illinois independently and with different groups of subjects, but with close knowledge of each other's experience, used a new method of correcting negative balance experiments by the assumption that about four to five times the amount of the negative balance would have to be added to the intake to find the true maintenance requirement. This assumption was based upon their findings of percentage "utilization" in the special sense in which they use the term. Computed in this way the data of Outhouse and her co-workers with seven subjects yielded an average of 0.75 gram, and the data of Steggerda and Mitchell with nine subjects showed an average of 0.67 gram, per 70 kilograms of body weight per day.

Three other studies, published in the period subsequent to

Leitch, are those of Owen, of Owen, Irving, and Lyall a little later, and of Chu *et al.* (1941). Combining the data of these three studies with those made at about the same time at the University of Illinois, as above described, gives a total of forty cases in five separate studies from three continents, which yield an average of 0.624 gram of calcium per man per day as representing the findings of the new experiments of the period 1939–1941 inclusive.

In the light of all the above evidence the National Research Council set its Recommended Dietary Allowance at 0.8 gram of calcium per man or woman per day for maintenance regardless of activity or occupation.

For women in the latter half of pregnancy the allowance is increased to 1.5 grams, and for those in lactation to 2.0 grams, of calcium per day.

Steggerda and Mitchell (1946*a*) bring together the data of new calcium-balance experiments as well as those that were contained in their 1941 paper cited above. The later experiments were largely for the purpose of studying the quantitative utilization of different forms of calcium by man; but were so arranged as also to afford further evidence on the calcium requirement of maintenance in healthy human adults. The average requirement of forty-three such subjects studied at the University of Illinois is reported by them as 0.65 gram of calcium daily, with a coefficient of variation of 22.5 percent. They recommended this average as "of value in the planning of diets." From this viewpoint it is in strikingly close agreement with the so-called dietary standard of 0.68 gram tentatively suggested on the basis of the data available in 1920, and largely used as a general guide from that time until the adoption of 0.8 gram as the Recommended Allowance published by the National Research Council in 1941 and 1945. The extent to which the more recent experimental evidence is more homogenous is indicated by its coefficient of variation of 22.5 percent as compared with a coefficient of 27 percent in the data available in 1920.

Leitch had held that the evidence showed no greater need of calcium in men than in women, and the National Research Council's Recommended Allowances provide 0.8 gram both for men and

women notwithstanding the fact that men average about one-fourth higher in body weight. According to Steggerda and Mitchell (1946, p. 416) it is probable that other factors are so much more potent in causing variation in calcium metabolism as to obscure the effect of variations in body size. They also suggest that 10 mg. of calcium per kilogram of body weight per day could be considered a well established approximation to the maintenance requirement of adults of the general food habits of American college students and staff members. An allowance half again as high as this would, on their calculation, cover the maintenance requirements of all but one or two people in one hundred.

This latter allowance would correspond to about one gram of calcium per person per day for healthy maintenance of adults. It is noteworthy that the extended studies of Leverton and Marsh (1942), described immediately below, also indicate that about one gram of calcium a day should be allowed for healthy adult maintenance.

In the writer's opinion this "standard" of one gram of calcium a day for healthy adult maintenance is preferable to any lower allowance.

Study of the data and discussion of Leverton and Marsh (1942) leaves one in some doubt as to how far their findings are the results of the nutritional needs, and how far of the food habits, of the young women whose calcium balances they report. They describe their investigation as a "study of metabolism on freely chosen diets," and these diets furnished "total average daily intakes (which) ranged from 0.421 to 1.679 g." of calcium per day in the ninety-eight studies of calcium balance which they record in a diagram of individual retentions plotted on calcium intakes, and in a tabulation of the numbers of studies and the average calcium-balance data for each of five groups divided according to range of daily intake as follows: 0.421–0.599, 0.600–0.799, 0.800–0.999, 1.000–1.199, and 1.200–1.679 grams of calcium. Of the first of these five groups, 61 percent showed negative calcium balances; and its average of calcium intake, which was 0.523 gram, may be taken as definitely below the average requirements of these young women. The second

group showed only 35 percent of negative balances, but its average intake of 0.703 gram fell short of its average output of 0.738 gram. According to one view the intake here might be considered as covering average requirements because, in a majority of cases, the calcium balance showed equilibrium or better. But Leverton and Marsh consider the negative average balance as meaning that a larger intake was needed, and this view seems to be supported by the data of the other groups. The group receiving 0.800–0.999 with an average of 0.896 gram of calcium a day showed average retention of 23 milligrams; that with average intake of 1.083 grams showed average retention of 89 milligrams; and that with average intake of 1.403 grams showed average retention of 134 milligrams.

Leverton and Marsh consider that "minimal requirement" for calcium can be read from the graph of their series as a whole as 0.83 gram per day and that this is confirmed by the finding that the cases showing equilibrium had an average intake of 0.875 gram per day. They add: "An intake of one gram, however, appears more desirable, for there is significant storage of the additional intake regardless of the age or nutritional background of the subject." Here there is an implicit assumption that increased retention is an asset and that this outweighs the consideration that some of the subjects of higher intake might have been able (if tested) to maintain equilibrium on less. In the opinion of the present writer, we should not be justified in extending this assumption of Leverton and Marsh to all nutrients; but it appears to be justified in the case of calcium, because of the very large amount of confirmatory evidence from animal experimentation which shows clearly and convincingly the long-run benefits of liberal calcium intakes.

CALCIUM REQUIREMENTS OF INFANTS AND CHILDREN

Jeans and Stearns have carried out, in systematic series, over four hundred balance experiments with infants in their first year of life.* These infants were fed whole milk with added carbohydrate and

* Reported to the Institute of Nutrition and the Society of Biological Chemists in 1938 and cited by Shohl (1939).

an adequate vitamin D supplement. Their intake averaged 135 mg. of calcium and 110 mg. of phosphorus per kilogram of body weight per day. Of this they retained about 50 mg. of calcium and about 30 mg. of phosphorus per kgm. per day. These figures are higher than hitherto accepted estimates of normal averages, but are similar to the estimates and recommendations of Leitch.

The Recommended Allowances of the National Research Council include provision for a sliding scale fitted to the body weight, so that rapidly growing and developing infants, such as Jeans and Stearns worked with, would be allowed more than smaller and slower-growing infants.

Calcium Requirements of Children. From the facts summarized in Chapter III it is clear that the normal growth and development of children requires increase not only of the amounts but also of the percentages of calcium in their bodies. As yet, however, there is not a clear-cut consensus of professional opinion as to the best rate of calcium retention in children at different ages. The work of Sherman and Hawley (1922) indicated that children from three to thirteen years show more advantageous retention of calcium when they have intakes of one gram of calcium per day than at any substantially lower level of intake. All the twelve children whom they studied by means of balance experiments with systematic stepwise increases showed higher gains at intakes of one gram than at intakes of three fourths this amount; but the extent of the difference between these two levels was subject to large individual variations from which it has sometimes been argued that only some, not all, of the children were importantly benefited by the increase of their calcium intake from 0.75 to 1.0 gram of calcium per day. That argument, however, neglects important evidence for, as we saw in the discussion of bone trabeculae in Chapter II, an extra storage of calcium, going largely or chiefly to an increased development of these trabeculae, does confer an important (even though unseen) benefit by making it easier for the blood to recover more quickly from any accidental downward fluctuation of its calcium content. The benefit thus de-

rived by the body from even a relatively small extra store of calcium has apparently not always been realized even by some of the research workers in the field.

Later investigations of children have indicated calcium allowances for them sometimes lower and sometimes higher than the familiar round figure of one gram a day, strongly supported by the experiments of Dr. Hawley. Dr. Amy Daniels published experiments which she interpreted as indicating a lower requirement, but Dr. Mary Rose showed, as described in Chapter III, that Dr. Daniels' experimental data were actually in close accord with Dr. Hawley's.

Leitch's compilation of the data of calcium-balance experiments with children regarded as normal by their observers showed a trend toward higher retentions than had been most commonly cited as average in earlier literature. She evidently regarded this modern trend to higher retentions as indicative of a more fully normal rate of development, and as supporting her view that the percentage of calcium in the normally developed body is higher than had previously been supposed. Shohl (1939) notes this difference, and while not fully displacing the earlier estimates by the later and higher ones, he seems to regard these latter as indicative of progress in the science of nutrition and the feeding of infants and children.

Some of Leitch's critics, however, have held all high retentions of calcium by growing children as "suspect," prejudging them all as presumably due to conditions of calcium depletion in the bodies of these children at the beginning of the balance experiments. Several of the papers from the University of Illinois tend to make use of this simplifying assumption, but it does not seem to be supported by the extensive studies made upon twelve children there by Dr. J. O. Holmes and her co-workers whose data are appended to the second volume of Macy's large monograph (1945). When twelve children, ranging in age from thirty-one to sixty-eight months, of reasonably well-known histories and presumably all normal, were fed from 1.5 to 1.86 grams of calcium per day through fairly long periods, to saturate their bodies as a preliminary to other studies, the balances shown on these high intakes varied too widely to fit (in the opinion of the present writer) into the simple assumption that they

reflected merely differing degrees of depletion and saturation in the children as they came into the experiment. This was undoubtedly one factor, but probably not the only one. Capacity to assimilate and use calcium appears from these data (as well as from x-ray studies of rates of skeletal development) to be too variable an individual characteristic to be explained wholly on the negative ground of a previously depleted condition. Rather these data suggest that there were also individual differences in the positive characteristic of capacity to assimilate liberal intakes and thus to make better than average progress in development toward skeletal perfection, and "chemical maturity" of the body as a whole. Hence the calcium intake allowance should be substantially above the merely average minimal need in order that the children of superior capacity may be provided with means to realize their full potentialities.

Undoubtedly too, the ideal, of supplying enough to enable each to realize his potential capacity, can be more confidently expected from moderate liberality consistently maintained, as in the National Research Council's Recommended Allowances of 1.0 gram per day or slightly over throughout childhood, than from sudden administration of unnaturally large intakes of over 1.5 grams a day. The extreme variations of calcium balance found by Holmes among children on such large intakes would seem to suggest that some of these children were (while others were not) in some way handicapped by use of so drastic a saturation procedure.

Yet notwithstanding the extreme variability of the data of the saturation periods, these data show that all of the twelve children made retentions of at least 200 mg. of calcium per day during at least some of these weeks of high calcium intake; and six of the twelve showed daily retentions of at least 300 mg. during at least some of their seven-day balance periods.

From these data it would seem safe to conclude that with abundant intake nearly any child should be capable of retaining 200 mg. of calcium a day, and that chances are about even that a given child can retain 300 mg. or more per day. In the experiments of Jeans and Stearns, retentions of 300 mg. of calcium per day appear to have been still more frequent. Individual differences have also been illus-

trated in another way elsewhere (Sherman, 1946*a*, pages 262–264).

After their thorough saturation treatment, the twelve children studied by Holmes were able to maintain equilibrium on intakes averaging about 0.35 g. of calcium a day, which is similar to the findings of earlier experiments by Hawley and by Wang and coworkers.

When Holmes thereafter increased the intake levels of her subjects to from 0.6 to 0.8 gram of calcium a day they showed retentions varying from 84 to 148 mg. and averaging about 115 mg. of calcium a day, which again is quite similar to the findings of earlier experiments with similar intakes.

The National Research Council's Recommended Allowance of 1.0 gram of calcium a day for children of an age range which more than covers Holmes' subjects, provides a reasonable margin of safety for physiological variability, and also makes possible the retention of somewhat more than 115 mg. per day by the children whose potentialities are somewhat higher than this.

In proportion as we now more fully realize the child's capacity to assimilate (for immediate or future use as the case may be) a good proportion of any surplus of calcium that the food may furnish, we see that instead of attempting overelaborate year to year adjustments of calcium allowances to the theoretical needs of a fluctuating growth rate, even better results may be expected from the simpler plan, recommended by the National Research Council, of an allowance of one gram of food calcium per day for all ages up to nine years inclusive, with moderately increased allowances between the ages of ten and twenty years. These intakes can easily be realized by use of natural foods which at the same time contribute importantly to the meeting of other nutritional needs.

Dr. Jeans and Dr. Stearns presented to the Society of Biological Chemists in 1938, and have kindly amplified by personal communications to the present writer, very comprehensive and consistent evidence of high calcium retentions under favorable conditions of liberal calcium intake, which could not reasonably be attributed to the children having previously been depleted by subsisting upon calcium-poor food.

Dr. Macy, summarizing the evidence of 593 calcium balances on twenty-nine children * studied in her own laboratories, reports (page 166 of Volume I of Macy's *Nutrition and Chemical Growth in Childhood*) that with intakes of 700–800 mg. per day the average retention was 125 mg.; and with intakes of 1700–1800 mg. the average retention was 696 mg.; while intermediate intakes resulted in intermediate retentions. Differently expressed, the data tell the same story of consistently increasing retention with increasing intake. Thus with calcium intakes of 20–40 mg. per kgm. of body weight, the average retention was 5.9–6.0 mg./kg.; with 40–50 mg./kg. ingested, 7.1 were retained; with 50–60 ingested, 18.2 were retained; with 60–70 ingested, 17.9 were retained; with 70–80 mg./kg. ingested, 24.2 mg./kg. were retained. The percentages of the ingested calcium which were retained ranged in Macy's groups on lower intakes between 12 and 26 percent; while in those with the higher intakes from 32 to 40 percent of the ingested calcium was retained. With such clear trends through such extensive data including many cases in which the same children served at the different levels, we cannot reasonably interpret the high retentions simply as due to previous shortages. Rather, we have here very strong confirmation of the body's surprisingly great ability to store surplus food calcium, largely as "working capital" in its trabeculae.

Macy's (1942, p. 13–15) work has also emphasized individual differences among children in their rates of skeletal development or the degrees of skeletal maturity attained at a given chronological age, and correspondingly in the efficiency with which they utilize calcium. Thus a boy whose skeletal development was far outrunning his chronological age retained almost twice as much calcium as did a child of the same age and calcium intake whose rate of skeletal development was much less. When both received the same amount, which was slightly less than one gram a day of food calcium, the child of rapid skeletal development retained an average of 469 mg. per day, while the other retained an average of 264 mg. per day.

It is, however, important to keep in mind that increased growth

* These children are described as "having lived healthy, well-ordered lives."

in body weight is not always accompanied by increased rate of skeletal development or of calcium utilization.

Macy (1942, p. 18–19) mentions the fact that Outhouse (Holmes) found an average of only 19 percent of a given calcium increment to be retained by children, and then states that in a similar experiment she (Macy) observed retention of 40 percent of the calcium increment, or fully twice as high an efficiency of utilization.

Certainly the trend of the evidence as to the amounts of calcium most conducive to good development in growing children was distinctly upward during the two decades following the experiments of Dr. Hawley, and this trend is to some extent reflected in the National Research Council's Recommended Dietary Allowances as first adopted in 1941 and readopted in 1945. These are as follows: For children up to and including nine years of age, 1.0 gram; for children 10–12 years, 1.2 grams; for girls 13–15 years, 1.3 gms.; for girls 16–20 years, 1.0 gram; for boys 13–20 years, 1.4 grams of calcium per day.

In the form in which the National Research Council has tabulated its Recommended Allowances, those for men are at the top, and those for teen-age young people are at the bottom. In a college class, however, there may well be boys over and under twenty years of age, whose food habits, size, and physical development are substantially the same. Hence the question legitimately arises among them: Does either the actual need or the optimal intake of calcium drop abruptly from 1.4 grams to 0.8 gram when the boy passes his twenty-first birthday? Obviously no scientific body intends to teach literally that! A large proportion of children now in their upper teens probably can advantageously use more than a gram a day of food calcium; yet if the children now being born are fed according to the Recommended Allowances and thus receive at least one gram of calcium throughout their first twenty years, they will, by then, have attained "chemical maturity" plus good body stores, and can thereafter be treated as adults. For adults, however, the allowance

of 0.8 gram calcium a day represents a less liberal margin for individual variation and for safety than the table of Recommended Allowances provides in cases of most other nutrients. Yet the experimental evidence of long-run benefits from liberal margins of intake of calcium is, as we saw in Chapter III, amply convincing. Hence a fully scientific use of present knowledge would keep the calcium intake up to at least one gram a day not only through childhood but well into or through adulthood as well.

Dr. F. J. Stare speaking at the 1943 Conference on Nutrition in Relation to Public Health, held under the auspices of The Nutrition Foundation, said of his recent hospital experience: "It was a surprise to see that the majority of x-ray studies on adults past the age of forty-five to fifty years showed considerable demineralization of bone, and one wondered if low dietary intake of calcium or of vitamin D, over many years, might not have been prominent factors in the etiology of this demineralization."

Such medical evidence reenforces the view that it is better not to let down calcium intakes so markedly at the age of twenty-one as the Recommended Allowances of the National Research Council would permit.

Another reason why liberality in the matter of dietary recommendations for calcium is scientific is that it provides a safety margin needed because of the unevenness of distribution of the nation's food supply among its people. Thus while the average consumption per capita (or per "nutrition unit") is about one gram of calcium per day in the United States, Stiebeling and Leverton (1941) found that in the Middle Atlantic and North Central region, 66 percent of the village family dietaries showed less than 0.68 g. calcium per nutrition unit per day.

Still another reason for liberality in recommending dietary allowances for calcium is the view that calcium in addition to its specific functions also serves the body as a sort of general regulator, as was especially emphasized by the late Dr. Samuel J. Meltzer, so that a dietary imbalance of some other factors may increase the body's expenditure of, and need for, calcium.

QUANTITATIVE ASPECTS OF THE PHOSPHORUS REQUIREMENT

Notwithstanding the many important functions of phosphorus compounds in nutrition, it has been generally assumed that, for the reasons outlined in Chapter I and aside from the special problem of low-phosphorus rickets, human dietaries adequate in other respects may be assumed to contain enough phosphorus; while no such assumption in regard to calcium is safe.

Studies of a quarter century ago indicated that the phosphorus requirement or turnover of adult maintenance averages about 0.88 gram per day (Sherman, 1920). The data were fairly consistent, showing a coefficient of variation of only 17. Men and women subjects were about equally represented and gave essentially the same results per kilogram of body weight. The literature since shows no substantial trend upward or downward. As a safe allowance, 1.32 g. per 70 kgm. of body weight has often been used. Dr. H. H. Mitchell, in a personal communication of March 6, 1945, to the present author, wrote regarding evidence available at that time: "My analyses of the data by a different method than yours led me to believe that the average requirements of calcium and phosphorus by the adult are respectively 9.75 and 16.66 mgms. per kgm. of body weight per day." On this basis the maintenance phosphorus requirement of a 70 kgm. adult would be 1.166 g. per day. This is in good accord with the view and practice of nutritionists generally in treating 0.88 g. as a "rock bottom" minimum requirement, and 1.32 as a desirable allowance for healthy adults, other than pregnant and lactating women, whose allowances are higher. Corresponding data indicate that to provide for a good rate of retention, children's dietaries should contain not less than about 1.0 gram of phosphorus a day (Sherman and Hawley, 1922).

The comprehensive mineral-balance studies of Jeans and Stearns, of Shohl (1939), of Macy-Hoobler (1941), and of Leverton and Marsh (1942) afford many illustrative data of the phosphorus intakes and retentions of children and young adults who were apparently developing normally. The last-named concluded from their

extended studies with young women of sixteen to twenty-five years of age that 1.06 grams of phosphorus a day may be considered the "minimal requirement," and 1.43 grams as the "optimal allowance."

In view of the recent great extension of our knowledge of the more qualitative aspects of phosphorus metabolism (Chapter IV) systematic quantitative investigation of the comparative effects of different levels of intake of various types of phosphorus compounds would now seem timely. In part, these might well parallel some of the experiments with different levels of calcium intake described in Chapter III and above in this chapter. Long-term studies of human subjects, with simultaneous attention to both calcium and phosphorus, might also yield results of much interest if the subjects could be kept under accurate observation throughout such lengths of time as would constitute considerable fractions of the normal life cycle.

True, the omission of a column for phosphorus in the National Research Council's Table of Recommended Dietary Allowances tends to emphasize the point of view that dietaries satisfactory in other respects may be assumed to be satisfactory in their phosphorus contents. This was a utilitarian simplifying assumption, made under wartime pressure for speed in formulating the allowances. Now, there may well come appreciation of the fact that even if permanently accepted as to mere minimal needs, it should not lead us to neglect the importance of the research problem as to how different intakes of the different kinds of phosphorus compounds may influence the long-run nutritional histories of entire lifetimes and successive generations.

DAILY TURNOVER OF BODILY ELEMENTS COMPARED

A daily metabolism of 0.88 gram of phosphorus, the average maintenance need for normal men as found above, may also be stated as a daily turnover of about one eighth of one percent of the phosphorus which the body contains.

Similarly the daily maintenance metabolism (exchange or turnover) of calcium in normal adult human bodies is about one twentieth of one percent, and the daily maintenance turnover of protein

is about one third of one percent of the amount contained in the body.

The fact that the turnover of protein is thus *relatively* about sevenfold more rapid than that of calcium is consistent with the fact that most of the body's protein is in its actively functioning tissues, while most of the body's calcium is held in the relatively inactive form of bone mineral. Compared with these, phosphorus has an intermediate distribution in the body and, consistently with this, an intermediate rate of turnover.

Thus, different as are the functions of these elements from some points of view, yet the nutritional need for each fits well into the general pattern of functional activity of all.

RATIOS OF CALCIUM TO PHOSPHORUS

The fact that distorted ratios of calcium to phosphorus played a large part in the production of experimental rickets led naturally to what might be called an invasion of the literature of these elements by an army of allusions to their ratios. From the viewpoint of normal nutrition it is well to remember that no perfection of adherence to any ratio can take the place of adequacy of intake of each of these elements, and also that when the intake both of calcium and of phosphorus is good the ratio between these elements must be good also.

Yet confusion sometimes arises from the fact that relatively more calcium is needed in the body's early *development*, and relatively more phosphorus in its later *upkeep*. Each of these facts, however, is simply explained, and, in fact, has been largely explained above and in previous chapters.

Normal calcification of the developing bones and teeth calls for deposition of a relatively large amount of bone and tooth mineral containing about twice as much calcium as phosphorus. Concentration of thought upon this calcification process has sometimes resulted in the suggestion of a calcium-to-phosphorus (Ca/P) ratio of about two (or 2:1) in the food of the young child. Such a view, however, loses sight of the fact that at the same time with the

growth and normal calcification (or ossification) of the skeletal system there is also growth of muscles and other soft tissues which contain (and require for their upkeep) much more phosphorus than calcium. Studies of the calcium and phosphorus balances of children with systematically varied intakes of these elements indicate that for best results the food should furnish not less than one gram each of calcium and phosphorus a day; which, of course, is a Ca/P ratio of one (1:1). The occasionally met recommendation that the calcium:phosphorus (Ca/P) ratio of children's dietaries should be between one and two (that is, between 1:1 and 2:1) is an attempt to combine the two viewpoints just considered. On the other hand, when the skeleton has reached the fully normal adult state of calcification, the need for calcium is relatively less. The maintenance requirements of normal adults tend, as we have seen, to average about six to seven tenths of a gram of calcium and about nine tenths of a gram of phosphorus per man per day. The ratio of these adult maintenance needs (or rates of turnover) is thus less than one. From the statistics of quantitative experiments upon the balances of intake and output by the normal adult human body, the Ca/P ratio of its respective needs appears as about 0.7; but this does not mean that the body is necessarily benefited by receiving less calcium than phosphorus. We have now seen that experimentation extended through entire lifetimes and successive generations of animals whose nutritional chemistry is, with respect to calcium, essentially like our own, shows generous margins of calcium intake to be very beneficial in the long run. As yet there do not seem to have been similarly comprehensive experiments at different levels of phosphorus intake. Hence the most scientific ground to take on this subject at present would seem to be that somewhat over one gram each of calcium and phosphorus is advantageous in human nutrition; and that with intakes of both these elements at this approximate level we have no occasion for anxiety as to the precise ratio between them.

If, in tables of calcium and phosphorus contents of foods, the Ca/P ratio is also given, this is chiefly as a familiar and convenient

rough general indication as to each food's relative richness in calcium, and does not mean that any particular ratio is, in itself and under all conditions, a point of superiority—for in this respect much will depend upon what other foods are being consumed and in what proportions.

Foods as Factors in the Nutritional Provision of Calcium and Phosphorus

THE title of this chapter is worded to convey the fact that some foods have properties, beyond their calcium and phosphorus contents, which bear upon the utilization of the calcium (or the phosphorus) that the dietary provides. Thus citrus fruits increase, and spinach decreases, the utilization of the calcium of the dietary as a whole. However, we here deal chiefly with the simple calcium and phosphorus contents of those foods for which these data are now fairly well established.

Here it seems most interesting to consider first the foods which are sometimes called "primary" or "direct food crops," and these in the order of the plant organs eaten, from roots and tubers, through bulbs, stems, and buds to fruits and seeds. Then, afterward, there follow the foods that are "secondarily derived" through animals. In each case we seek an adequate number of important illustrations with no attempt to be all-inclusive. For, users of this book will presumably have at hand the Government bulletin giving simple average values for practically all kinds of foods. This chapter is therefore devoted to fewer foods, and these are so given as to show their variability and the degree of precision of our knowledge of them.

Table 8 presents the averages of available evidence on the calcium and phosphorus contents of certain edible roots, tubers, bulbs, and stems. In accordance with the usage of the United States Department of Agriculture and the National Research Council * the data are given in terms of milligrams (mg.) of calcium and of phosphorus, respectively, in 100 grams of the edible portion of the food, usually in the condition in which it passes from the dealer to the consumer.

* U.S. Dept. of Agriculture, Miscellaneous Publication No. 572, (1945).

TABLE 8

Calcium and Phosphorus Contents of Roots, Tubers, Bulbs, and Stems

KIND OF FOOD	CALCIUM			PHOSPHORUS			
	No. of Cases	*Mean ± its P.E. mg. per 100 g.*	C.V.	*No. of Cases*	*Mean ± its P.E. mg. per 100 g.*	C.V.	*Ratio Ca/P*
Tap roots							
Beets	45	24 ± 1.2	47	68	41 ± 1.1	32	0.59
Carrots	50	42 ± 1.0	26	47	40 ± 1.2	32	1.05
Parsnips	22	44 ± 1.2	15	19	75 ± 2.7	23	0.59
Radishes	22	37 ± 2.5	47	14	33 ± 1.6	28	1.12
Turnips	36	45 ± 2.1	41	155	32 ± 0.06	36	1.40
Cluster roots							
Sweet potatoes	34	30 ± 1.6	45	34	53 ± 1.8	29	0.57
Tubers							
Potatoes	300	9 ± 0.21	61	135	56 ± 0.8	26	0.16
Bulb							
Onions	43	38 ± 1.1	28	67	33 ± 1.1	39	1.15
Stem with Growing Tip							
Asparagus	27	20.3 ± 1.0	39	21	53 ± 2.4	31	0.38
Stem only							
Celery	22	56 ± 3.0	37	33	42 ± 1.2	25	1.33

From Table 8 it appears that the differences between typical roots, tubers, bulbs, and stems, in their calcium and phosphorus contents are not very pronounced except for the low calcium content of potatoes and the (less pronouncedly) high phosphorus content of parsnips. The calcium:phosphorus ratios range from 0.4 to 1.4 except that the low calcium content of potato gives it a ratio of only 0.16.

Table 9 gives corresponding data for typical edible leaves and buds. Several of these will be seen to be so high in calcium (and in Ca/P ratio) as to be very efficient in balancing or supplementing potato and breadstuffs in this respect. The high calcium contents of broccoli, collards, kale, mustard greens, and turnip greens make them important factors in the nutritional supply of calcium. This becomes still more striking when we consider the high moisture content of these leaves.

Mallon and Urey (1941) found, both in California and in New York lettuce, that the calcium content was two to three times as high in the outer as in the inner leaves. Outer leaves showed 0.2 to 0.3 percent, and inner leaves 0.09 to 0.11 percent of calcium in the fresh substance. Cowell had found still greater differences between the inner and outer leaves of headed cabbage.

As Dr. J. O. Holmes has put it, it is probable that few, if any, other foods yield their calcium to man to a greater extent than does milk. In her own experiments about twice as high a proportion of the calcium of milk as of carrots was utilized by seven human adults.

In other experiments, growing animals retained, under like conditions, 80 percent of the calcium of milk, 71 percent of that of carrots, 61 percent of that of lettuce, and 60 percent of that of snap beans (Shields, Fairbanks, Berryman, and Mitchell, 1940).

Fincke found that the utilization of the calcium of broccoli and of kale was about nine-tenths as high, and that of cauliflower about eight-tenths as high as that of milk; while the calcium of spinach was nonutilizable, under the same conditions. (Conditions can be so arranged as to indicate slight utilization of calcium from spinach;

TABLE 9

Calcium and Phosphorus Contents of Edible Leaves and Buds

KIND OF FOOD	CALCIUM			PHOSPHORUS			
	No. of Cases	*Mean ± its P.E. mg. per 100 g.*	*C.V.*	*No. of Cases*	*Mean ± its P.E. mg. per 100 g.*	*C.V.*	*Ratio Ca/P*
Broccoli	23	179 ± 15.2	61	14	72 ± 2.3	18	2.5
Brussels sprouts		34 [a]			78 [a]		0.44
Cabbage	118	52 ± 1.3	46	103	30 ± 0.6	32	1.7
Cauliflower	22	24 ± 0.75	22	23	66 ± 2.1	23	0.36
Collards		249 [a]			58 [a]		4.1
Dandelion greens	8	113		8	43		2.6
Kale	51	250 ± 6.0	25	51	78 ± 2.1	28	3.2
Lettuce	82	33 ± 1.3	54	83	28 ± 0.9	43	1.2
Mustard greens		220 [a]			38 [a]		5.8
Turnip greens	114	272 ± 4.3	25	114	59 ± 0.6	17	4.6
Watercress	12	169 ± 7.4	22	14	39 ± 2.7	39	4.3

[a] U.S. Dept. of Agriculture, Miscellaneous Publication 572 (1945).

but the weight of the evidence is now quite clear that the nutritive value of spinach calcium is negligible, or negative.)

Speirs found the calcium of turnip greens almost as available as that of milk, while the calcium of collards and kale was only slightly less well utilized.

The leaves of plants of the goosefoot family (Chenopodiaceae) which includes beets, chard, and spinach, are not included in the table here given because their calcium is practically (or essentially) *not nutritionally available*. Doubtless this fact is chiefly due to the relatively large amounts of oxalic acid which these leaves contain. The phrase which one sometimes meets to the effect that the calcium in these leaves, "may not be available because of presence of oxalic acid" is, in the opinion of the present writer, so extreme an understatement as to be misleading. Natural variations will, of course, result in somewhat differing ratios of calcium to oxalic acid in different specimens; but, in the case of spinach at least, the evidence of research findings is now so strong and clear as to be fully convincing to anyone who is scientifically trained and open-minded. We should all admit that spinach was a mistaken choice for popularization as a typical green-leaf vegetable, and that it now should be replaced by green leaves of other botanical families such as broccoli, loose-leaf cabbage, collards, dandelion greens, kale, loose-leaf lettuce, mustard greens, turnip tops, and watercress. Circumstances have made the writer the recipient of probably about all of the objections to the replacement of spinach by better greens, and these objections add up to nothing more than an unscientific reluctance and inertia about making a reasonable adjustment to new knowledge. Every grower, and canner, and teacher, on looking around open-mindedly, will find practicable alternatives among the greens that are nutritionally superior to spinach.

Noteworthy among the facts summarized in Table 9 are the high calcium contents (and high calcium:phosphorus ratios) of the green leaves representing at least three botanical families. As suggested above, if the low calcium content and calcium:phosphorus ratio of potatoes as shown in Table 8 has caused anxiety, note how efficient a balancing food in this respect one may choose (according

to one's preference) from among the edible green leaves shown in Table 9.

Usually the calcium content of a food chiefly determines the value of that food as a factor in the nutritional supply of calcium to the body. In some cases, however, there are modifying factors, some of which decrease and some of which increase the obvious implications of the calcium content. Thus we saw that the green leaves of spinach contain so much oxalic acid as to reduce, practically to nil, the value of spinach as a factor in our nutritional calcium supply. On the other hand, orange juice increases the nutritional availability of the calcium in the dietary by a larger amount than its own calcium content. This fact has been consistently shown both by calcium-balance experiments with children and by experiments with growing animals in which calcium retentions were determined by analyses of the bodies of the animals as well as of their foods (Chaney and Blunt, 1925; Lanford, 1939). Mallon and Rosenblatt (1946) find grapefruit juice like orange juice in this respect, while tomato juice did not show this advantageous property in their experiments.

Sherman and Ragan (1942) found that a large part (averaging about 73 percent) of the calcium of a peeled orange is contained in the fibrous *septa*, as distinguished from the juice and also from the softer less fibrous part of the pulp which is removed with the juice in varying proportions according to the method of reaming or pressing employed, and whether (and if so how) the juice is strained. Thus the whole edible portion of orange, as served sliced, is considerably richer in calcium than is orange juice; and the juice as served is lower in calcium content the clearer it is.

Calcium and phosphorus contents of a wide range of fruits are shown in Table 10.

Table 11 shows corresponding average data for calcium and phosphorus in the edible portion of the snap or string bean (pod with immature seed); and then in a number of mature seeds of widely scattered botanical origin. It will be seen that with few exceptions the seeds we use for food, and the breadstuffs made from them are of much lower calcium than phosphorus content. White flour contains much less of both these elements than does the wheat from

TABLE 10

Calcium and Phosphorus Contents of Fruits

KIND OF FOOD	CALCIUM			PHOSPHORUS			
	No. of Cases	*Mean ± its P.E. mg. per 100 g.*	C.V.	*No. of Cases*	*Mean ± its P.E. mg. per 100 g.*	C.V.	*Ratio Ca/P*
Apples	110	7.4 ± 0.25	52	82	11. ± 0.17	21	0.67
Avocado	42	19. ± 1.3	66	67	53. ± 1.9	44	0.36
Bananas	32	6.7 ± 0.3	39	33	28. ± 0.8	24	0.24
Cantaloupe	19	15.8 ± 0.9	37	18	18. ± 0.95	33	0.88
Cherries	20	18.2 ± 1.0	37	14	24.5 ± 1.1	26	0.74
Cranberries	13	13.3 ± 0.5	21	13	10.2 ± 0.4	21	1.30
Dates (dried)	15	72. ± 2.7	22	16	60. ± 2.1	21	1.20
Orange, entire E.P.	19	43. ± 0.8	12	15	21. ± 0.5	13	2.05
" juice, pressed	22	8.2 ± 0.23	20	11	17. ± 0.4	12	0.48
" " misc.	34	15.1 ± 0.6	36	20	15.4 ± 0.5	23	0.98
Peaches	19	9.0 ± 0.8	55	19	18. ± 0.55	20	0.50
Pears	17	12.4 ± 0.7	36	15	15.2 ± 0.9	35	0.82
Plums	23	15.3 ± 0.9	43	16	19. ± 1.1	35	0.80
Watermelon	10	7.4 ± 0.84	53	10	11.8 ± 1.06	42	0.63

TABLE 11

Calcium and Phosphorus Contents of Seed Pods, Seeds, and Breadstuffs

KIND OF FOOD	CALCIUM			PHOSPHORUS			
	No. of Cases	*Mean ± its P.E. mg. per 100 g.*	C.V.	*No. of Cases*	*Mean ± its P.E. mg. per 100 g.*	C.V.	*Ratio Ca/P*
Pod with immature seed							
Beans, snap or string	53	65 ± 1.8	31	37	44 ± 1.3	26	1.48
Seeds							
Almonds	31	254 ± 7.8	26	27	475 ± 14.1	23	0.53
Beans, dry	29	148 ± 4.9	26	32	463 ± 6.6	12	0.32
Lima, dry	19	73 ± 1.4	13	21	380 ± 0.6	11	0.19
Corn (maize), mature	31	21.7 ± 2.78	106	61	310 ± 0.4	15	0.07
sweet, fresh	21	8.5 ± 0.61	49	18	120 ± 1.8	9	0.07
Oats (oatmeal)	17	55 ± 1.4	15	18	395 ± 14.9	24	0.14
Peas, dry	63	73 ± 1.6	26	65	376 ± 6.8	22	0.19
fresh, green	47	25 ± 1.0	40	32	132 ± 3.1	20	0.19
Wheat, entire	94	57 ± 1.3	32	70	374 ± 4.3	14	0.15
White flour	23	15 ± 0.7	33	25	101 ± 2.1	15	0.15
White bread	30	31 ± 2.0	42	23	97 ± 2.0	14	0.32

which it is made. White bread, with about the same phosphorus content as white flour, has about twice as much calcium as has the flour because of the milk now commonly used in breadmaking and the calcium salts often added to favor the activity of the breadmaking yeast.

Table 12 shows the corresponding calcium and phosphorus data for some of the chief foods of animal origin. The calcium content of milk is about twice that of eggs, and exceeds that of meat in still higher degree. Although, as may be seen from comparison of data in Tables 9 and 12, some of the green-leaf vegetables have higher percentages of calcium than has milk, yet because of the quantity in which it is commonly and wisely used in the United States and many other western countries, milk becomes the main source of calcium in our dietaries. It is therefore important to find that milk is also a very reliable source of both calcium and phosphorus, the coefficient of variation of its calcium and phosphorus content being only 7 for each of these elements.

As shown by the investigations of McCammon, Caulfield, and Kramer (1933), rennet cheeses such as those of the Cheddar type retain about four fifths of the calcium of the milk from which they are made, while the acid types of cottage cheese retain only about one fifth. Correspondingly, the percentage of calcium in the finished product is about ten times higher in Cheddar cheese than in acid cottage cheese.

Dried skimmed milk (defatted milk solids) is rich in calcium, averaging about 1.33 percent. Its use in breadmaking adds importantly to the calcium, protein, and riboflavin value of the bread. It can also be used in cookery to improve the nutritive values of many other foods.

Lean meats show a fairly uniform high content of phosphorus, while their calcium content is low and (according to published analyses) extremely variable. The variability of the calcium content of muscle meats is probably largely accidental, and of no great practical importance to food values because meat (at least as ordinarily prepared and eaten) makes only a negligible contribution to the dietary intake of calcium. In some other countries more of the cal-

TABLE 12

Calcium and Phosphorus Contents of Foods of Animal Origin

KIND OF FOOD	CALCIUM			PHOSPHORUS			
	No. of Cases	*Mean ± its P.E. mg. per 100 g.*	C.V.	*No. of Cases*	*Mean ± its P.E. mg. per 100 g.*	C.V.	*Ratio Ca/P*
Milk	274	118 ± 0.37	7	214	93 ± 0.3	7	1.20
Cheese, Cheddar type	41	803 ± 19.0	22	32	524 ± 18.0	28	1.53
" cottage type	22	95 ± 2.8	20	14	181 ± 12.0	38	.52
Eggs	36	58 ± 1.0	16	104	224 ± 1.4	10	.26
Beef, lean	45	13 ± 0.7	56	40	204 ± 2.5	11	.06
Other lean muscle meats	47	14.7 ± 0.7	50	37	214 ± 3.9	16	.07
Oysters	11	70 ± 8.3	59	11	170 ± 8.5	25	.41

cium of the bones is brought into the meat in such ways as described by Hoh and co-workers (1934) and by J. C. Williams (1936). American Indians accomplished this also. In Mary Rowlandson's account of the food habits of the New England Indians by whom she was held for ransom in 1676, it is said that they would cut bones in pieces at the joints, boil them and drink the liquor, and then pulverize the ends of the bones in a mortar and eat the bone meal thus obtained. More recently, LaFarge, in an account of a feast made by Indians of the Southwest, speaks of long cooking of corn with beef bone in water. As we now know, this would carry a significant amount of calcium from the bone to the cooking water and the softened absorbent corn.

The United States Bureau of Fisheries reports that the calcium of the bone of canned salmon is nutritionally available when the bone, which has been cooked along with the flesh and thus softened in the can, is consumed with the flesh as human food. From the Orient it has also been reported that the bones of small fish, eaten by man, may be counted as digestible and their calcium as available, regardless of the method of cooking.

Allowing for the differences due to fat and to bones, fish, like meats, have high phosphorus, and lower, but much more variable, calcium contents.

In a large majority of the comparisons afforded by the tables in this chapter (38 out of 50 foods) the variations in reported contents are greater with calcium than with phosphorus. This may, in part, be a misleading appearance due to relatively larger errors in sampling and analysis in the case of the calcium. Probably, however, the difference can only in lesser part be thus explained away; and if calcium contents of foods are, in fact, more variable than their phosphorus contents, we have here an added reason for so planning dietaries and food supplies as to furnish distinctly liberal margins in food-calcium content over the estimated nutritional needs.

From even the simple statistical treatment of the data for average composition in the accompanying tables, one may see that, for many of the foods which are important sources of these elements,

knowledge of the average calcium and phosphorus contents has now reached a good degree of dependability.

Other Causes of Variations in the Calcium and Phosphorus Contents of Foods. As explained in Chapter I, plants tend to maintain their highest calcium contents in their green leaves, while tending to concentrate their phosphorus in their seeds.

In cultural varieties, however, the characteristics which have been fixed by breeding may modify the composition from that of the original natural form. Thus our common cabbage with its tight head of nearly white leaves has lower calcium content than has loose-leaf cabbage with its much greener leaves, or than have the green leaves of broccoli, collards, kale, or turnip tops.

Again, the edible portion of broccoli contains three organs of the plant: flower buds, adjacent green leaves, and the tender twigs on which both the buds and the leaves are borne. Calcium and phosphorus contents are both different for the three parts, so the amount of each element one gets from this food depends upon the proportions in which the three parts are eaten. The descending order of calcium content is leaves, buds, twigs; that of phosphorus content is buds, leaves, twigs.

Moreover, even in the case of a given variety of turnip tops which does not present the complications of the preceding two paragraphs, the variations from sample to sample were very considerable and as yet have been only in part accounted for by studies of the effects of fertilizers, soil composition, and climatological conditions (Sheets, McWhorter, *et al.*, 1943).

Sometimes the composition is materially changed by established processing practices. Thus white flour is lower, in both calcium and phosphorus content, than the wheat from which it is milled. Yet bread is (if compared on the same basis of moisture content) higher and more variable in calcium content than the flour from which it is made, because of the calcium of milk used in breadmaking and the salts introduced as "yeast food."

The relative constancy of the calcium and phosphorus contents

of milk, and of the phosphorus content of lean meat, have been noted above.

Much further research will be required before the influence of various environmental conditions and the varietal differences within a given kind of food can be summarized comprehensively and with confidence. The general situation with reference to vitamin values as well as mineral elements has been discussed elsewhere (Sherman, 1946*a*, pages 561–579).

CHAPTER VII

Summary and Suggestions

In this concluding chapter our purpose is to pass in brief review some of the chief facts and principles which have been developed in the previous chapters, but here in a somewhat different sequence, to facilitate seeing the facts from different angles of approach, and with such additional interpretation as can best be undertaken in the light of the story as a whole.

Calcium and phosphorus are the two chemical elements which stand highest in a quantitative accounting of the mineral composition of our bodies (aside from their moisture content). Each presents a significant nutritional problem in the sense that either of them may become the *limiting factor* in the general nutritional process of the body, thus actually determining what occurs in our lives or those of our children, as well as of animals both in nature and under domestication.

Shortages of phosphorus occur the more frequently with herbivorous animals, and perhaps with infants whose rapidly growing muscles may in effect be competing with their skeletal systems for the available phosphorus (phosphate ions) brought by the blood. Low-phosphorus rickets was therefore frequent until recently; but now that such rickets is generally prevented by the combination of better diet and vitamin D, calcium is probably more often the limiting factor with infants as well as with older children.

Thus, as we saw in Chapter V, the National Research Council offers a specific Recommended Dietary Allowance for calcium at each age period; but does not provide a complete parallel column of recommendations for phosphorus. In place of the latter, it is stated that dietaries of adequate protein and calcium contents may be trusted (without further planning) to provide adequate phosphorus also. This is probably a safe assumption wherever milk enters

as largely into the dietary as it now usually does in the Western World, because milk is an excellent source of phosphorus as well as of calcium.

That phosphorus content may be presumed adequate in dietaries of adequate protein and calcium content is, however, obviously a utilitarian simplifying assumption. Hence it is to be expected that as peacetime conditions permit of more deliberate research, and under the stimulus of the great recent advances of qualitative knowledge summarized in Chapter IV, there may also soon come a renewal of interest and of research activity in the quantitative aspects of phosphorus metabolism for which development Chapter IV attempts to indicate starting points for study of the essential background. That chapter is itself so concisely constructed as hardly to permit of again abstracting here.

However, it may well be pointed out here that the principle of the nutritional improvability of the norm, now so well established by recent and current research upon the full-life and successive-generation effects of different liberal levels of calcium and of vitamin A, strongly suggest similar experimental studies of dietaries so constructed as to contain different kinds and amounts of food phosphorus. Hence the tabulations of data in Chapter VI give the same attention to the phosphorus as to the calcium contents of foods.

The outstanding feature of the calcium problem in infancy is that human infants, as well as those of many others of the higher animal species, are born calcium poor. This means that the body of the newborn baby contains, not simply such smaller amount than that of the adult as would be expected from the difference in size, but also that the infant contains a smaller percentage of calcium. Hence the normal development of infants and children requires food supplies relatively richer in calcium than in body-building elements in general, in order that the body may increase both its size and its percentage of calcium at the same time, and both at a fully normal rate.

When we seek to give this latter concept a precisely quantitative form, corresponding to our ideal of developing the chemistry of nutrition into the status of an exact science, we find that the de-

scriptive knowledge involved is not equally precise at all points.

The evidence is quite consistent that the normal human infant contains at birth about 20 grams of calcium, or about seven tenths of one percent of its weight.

The fully developed human adult can safely be said to contain more than twice this percentage of calcium; but, as to just how much more, there is not yet a quantitatively exact consensus of opinion even among those who have given special study to the subject.

This doubt as to the calcium content of the optimally developed human adult must necessarily throw a corresponding doubt upon any attempt to deduce standards for calcium requirements, or retentions, or body composition at different ages, from any assumed standard percentage of calcium in the adult human body.

Both from the viewpoint of obtaining a scientifically sound picture of the normal developmental process of calcification in quantitative terms, and as an assembling of facts to guide the professional work of human nutrition, the best plan seems to be to start with the above-mentioned average newborn baby containing about 20 grams of body calcium and to study the evidence as to the gains to be expected, desired, and fed for, at successive ages and stages of development.

For infants in their first year, and for children of from one to three, four to six, and seven to nine years, the Recommended Allowance * is, in each case, 1.0 g. of calcium per day. Here the evidence for each of these four age periods was considered separately and on its own merits. The fact that the National Research Council and its Food and Nutrition Board decided, on the basis of all available evidence, to recommend the intake of 1.0 gram of calcium a day for each of the four age periods which together cover the first nine years of life is not only a great practical convenience; it is also a fact of much scientific significance.

If, in accordance with this recommendation, an average child receives 365 grams of calcium in his food in the course of his first year of life, we may expect that about 20 percent of this or about 73

* These two words capitalized stand for the Recommended Dietary Allowances of the Food and Nutrition Board of the National Research Council.

grams of calcium will have been added during the year to his initial 20 grams of body calcium. As the ratio of increase is thus larger with calcium than with body weight, the body will have increased not only its amount but also its percentage of calcium during its first year.

If, in successive years, the daily intake remains at one gram and the retention at about 200 milligrams, the body, while gaining the same arithmetical amount, will increase its percentage of calcium by successively smaller increments and, in a few years, will reach the condition of so-called chemical maturity, where further gains in body weight and in body calcium are about proportional to each other, the percentage of calcium in the body having thus been brought up to the normal adult plateau value.

This gives us, along with the practical guidance to good nutrition, a mental picture of the chemical aspect of skeletal maturation in the growing child, of the essential correctness of which we have abundant evidence, even though we have not yet a settled consensus of expert opinion as to the exact altitude of the "plateau value" of the percentage of calcium in the adult human body.

X-ray studies confirm the general pattern of the picture afforded by the chemical evidence. Precise parallelism in all cases is not to be expected. Ossification as judged from roentgenograms, and calcium retentions as found by chemical determinations of intake and output of calcium by the body, are both subject to individual variations, which, however, do not seriously impair the validity of the general concept which is afforded by the study of the evidence as a whole.

The question may arise whether the Recommended Allowances, and this discussion of them, need more elaboration because of the fact that the rates of bodily growth and skeletal development are not uniform throughout the first nine years of life. True, these rates of growth and development show some fluctuations, but these fluctuations do not come at the same ages in all children. Hence, it is probably more scientific to provide a liberal allowance at all times and trust the body to store its surplus for use at any future time of need. This position is strongly reenforced by the recent advances

in our knowledge and appreciation of the bone trabeculae as readily available reserve stores of calcium for use by the body.

Returning to the Recommended Allowances, we find that (in recognition of the needs of their adolescence) more than the above-discussed one gram of calcium per day is allowed for girls of ten to fifteen, and for boys of ten to twenty years (girls of sixteen to twenty being again allowed one gram a day). At twenty-one years of age, the Recommended Allowances for both girls and boys drop to the adult maintenance allowance of 0.8 gram per day for both men and women, with provision for 1.5 grams for women in the latter half of pregnancy, and 2.0 grams during lactation.

It is probably better to allow 1.0 gram (instead of 0.8 g.) for adult maintenance, in order to provide more fully for the covering of individual variations of calcium need among people. Also, variations of available calcium content among some types of food is an additional reason for the more liberal allowance.

Chiefly, however, the more liberal allowance is advocated because recent research shows much more forcibly than was known or suspected before that (*a*) some people, without bearing any sign of the fact, actually need considerably more calcium than the average on which the Recommended Allowances are based, and (*b*) the health of all should be safeguarded by providing considerably more than the directly demonstrable need.

The outcome of long study of the large amount of evidence now available may be summarized in a single sentence as follows: One gram of food calcium is a desirable part of the daily food supply of everyone, with probably somewhat more in the years from ten to twenty, and certainly substantially more in the latter half of pregnancy and during lactation.

This simple general recommendation is in entire agreement with the detailed Recommended Allowances of the Food and Nutrition Board of the National Research Council, with the added insurance of a somewhat more liberal allowance (than that of the Council) to be for the best interests of adult maintenance.

The reason for this difference of opinion is that the Council recommendation for maintenance is fitted rather closely to the

average finding of requirement in the sense of the calcium-balance experiment, whereas, in view of the individual variations of need, as shown by these experiments, and the benefits of liberal calcium intakes in large numbers of full-life experiments with laboratory animals, the writer considers the higher maintenance allowance here recommended as reflecting somewhat more adequately the evidence as a whole, and as providing a margin for safety more nearly proportionate to those of the Council's recommendations regarding most other nutrients.

The general intention of those making dietary allowances, and the general expectation of those who use them, is that the recommended allowance of each nutrient for each category of persons shall cover the needs of substantially all the normal persons of the category. Or in other words, the recommendation should be set at a sufficiently high level to cover all the individual variability of need (for the nutrient in question) that one is likely to meet in practice. Plainly this means something distinctly higher than merely the mean (average) requirement.

The recent extended investigation of Leverton and Marsh (1942) and the still more recent critical study and statistical interpretation of calcium balances by Steggerda and Mitchell (1946) both indicate that an allowance for adult maintenance set at a level to cover ordinary variability (covering, for example, the needs of all but one or two people in one hundred of a passably healthy population) is about 1.0 gram of calcium per day for adult maintenance.

On scientific grounds, the percentage of margin for safety and for individual (physiological) variability by which a recommended dietary allowance shall exceed the average directly demonstrable need should be different for different nutrients or nutritional factors. The need for such discrimination may not yet be recognized by all who have to do with such recommendations, but should be for at least two reasons:

(1) The actual percentage variability among ostensibly normal people of a given category of age, sex, size, and occupation is greater with respect to some nutritional factors than to others.

(2) Of some of the essential factors in our nutrition our bodies

can *advantageously* store large percentage surpluses above their immediate needs, while of others anything more than a small percentage surplus is wasted or may be burdensome to the body.

For both these reasons a dietary or food supply scientifically planned to make full use of nutritional knowledge will provide relatively larger surpluses of calcium than of calories. Of some other nutrients, notably vitamin A, we can say the same as of calcium in this respect; of still others we want only small surpluses as with food calories; and of most nutrients including individual amino acids we are not yet (1947) prepared to say what margin of surplus above actual need is desirable. Long-time experiments (with large numbers of well-standardized animals) upon different levels of intake, such as have been reviewed for calcium (Chapters II, III, and V) and suggested above for phosphorus, may well be suggested also for each nutritionally essential substance, including individual amino acids.

The effects of food upon health and vitality can now be studied by means of recently developed techniques with results much more conclusive than were possible previously. For nutritional experimentation has been promoted to the status of the exact sciences, with correspondingly increased objectivity and convincingness. We have learned, through experimental study, enough about both the differences and the resemblances between certain species in their nutritional chemistry so that we now know which of the practicable species are eligible to serve as our deputy subjects in the study of problems of human nutrition, according to the chemical aspect of the nutritional process which is involved in the problem in hand. Then, with a laboratory-controlled colony of appropriate species, from which standardized experimental animals of known hereditary and nutritional backgrounds can be drawn at precisely known ages, grouped in strict parallel, and fed the diets to be compared throughout entire lifetimes and successive generations, in sufficiently large numbers for conclusive statistical analysis of results, one has a research instrument of precision of a kind which has never been available before. And, correspondingly, the findings of such research in nutrition and food values are now moving from the realm of opin-

ion to that of fully established and unquestioned scientific fact.

Thus studied, the long-run effects of liberal, as distinguished from merely minimal-adequate levels of food calcium, are found to include at least the following benefits: (*a*) A more efficient use of the food, e. g., more growth per unit of protein or per 1,000 calories of food consumed. (*b*) A better rate of development so that full adult capacity is somewhat more promptly attained. (*c*) A higher general level of adult vitality—visible and palpable in the experimental animals, objectively measured in the records of their offspring—with the human implication of greater fitness for whatever occupation may be pursued. (*d*) A longer "period of the prime" between the attainment of maturity and the onset of senility, meaning for human life a smaller percentage of years of dependence and a greatly improved prospect for the attainment of life's ideals. (*e*) Lower death rates at all ages with consequent improvement of the life expectation both of children and of adults.

Stepwise study of the effects of different degrees of liberality of food calcium revealed a further fact of marked interest both in itself and as illustrating how improved comprehensiveness and precision of animal experimentation may clarify and strengthen its interpretation.

In a two-generation comparison of the effects of moderately differing adequate * levels of calcium in the food of animals strictly parallel in other respects, all the benefits just summarized were measured and found to be statistically significant with the single exception that the average length of life of the females was apparently only insignificantly increased by their (moderate) increment of food calcium. This fact raised two questions, namely: Were the females less able than the corresponding males to invest extra food calcium in an additional length of life? Or, inasmuch as these females had produced and reared more and sturdier offspring than their sisters on lower-calcium food supplies, should the facts be

* The lower of the two levels here studied was adequate to the support of entirely normal life cycles generation after generation. Families are thriving on it in the 63rd generation at time of writing (January, 1947), yet they appear to have lost nothing of the ability to make good use of higher levels of calcium content in their daily food.

interpreted as meaning that they had invested their extra food calcium in posterity instead of in longevity?

To answer the first of these questions, full-life comparisons of females on the two diets differing only in calcium content, were made with *unmated females*. Here, there being no opportunity to invest the extra calcium in offspring, the females on the higher-calcium diet showed very significantly longer life than their twin sisters on lower-calcium food supply. Then in another series, the difference in calcium content of diet was *increased* and the families on both calcium levels were allowed to reproduce without restriction. Here the females having the *increased amount of extra calcium* showed superior life histories in both ways: they reared more and sturdier offspring *and* they enjoyed increased longevity themselves.

Thus these experiments afford two independent demonstrations —both by entirely objective methods and criteria—of enhanced vitality induced by extra food calcium even though the calcium of the original dietary was already adequate to the support of normal nutrition generation after generation indefinitely.

From the chemical viewpoint the facts are seen to be satisfactorily explained by the physico-chemical structure and functioning of the bone trabeculae, and by the state of calcium in the blood (Chapter II).

Hence, also, the significance of the fact that extra calcium is beneficial to vitality and life history is not impaired by failures to find differences in blood-calcium concentration. The extra calcium may function in some other way, or a difference in the concentration of calcium or calcium ion in the blood may be too small for measurement by present analytical methods and yet may have very real effects in the course of a lifetime.

One of the greatest benefits offered by the newer knowledge of nutrition is in its firm establishment of the principle clearly taught by McCollum that there is, or may be, an important difference between the merely adequate or passable and the optimal in nutrition. Both for public health and for individual well-being and efficiency this means, as J. F. Williams has well emphasized, that health is not merely the absence of disease; but, rather, it is a positive quality

of life which can be built to higher levels. This concept of an attainable higher health which is well above the level of the merely adequate or passable, is one of the most important of recent scientific advances. Undoubtedly one of its greatest significances is in and through nutrition. And here it can and should mean something much more than simply a habit or an attitude of indiscriminate openhandedness with all nutrients. For the width of the zone between the level of minimal adequacy and the optimal level is, as we have seen, very different for the different nutrients or nutritional factors.

It is also to be remembered that, as noted elsewhere, extended pathological investigation has shown that doubling a normal food-calcium intake did not increase the incidence of abnormal calcification in the body, as of arterial walls or other soft tissues; whereas surplus intake, for example, of cholesterol may not be so harmless.

In the working out of the relations of calcium to nutrition through feeding experiments such as have been outlined, the extra calcium was usually given as one or another pure salt of this element in order to be sure that no effect of any other element should influence the effects attributed to calcium. In the practical enrichment of the calcium content of dietaries and food supplies, however, it will rarely, if ever, be advisable to use anything so artificial as these pure salts. Natural foods of high calcium content are normally a much more desirable source of calcium than mere calcium salts, because in the natural food one gets other valuable nutrients along with the calcium.

As McCollum emphasized so effectively, there are two types of food that are relatively rich in both calcium and vitamin A value. These are the green-leaf vegetables and milk. Today we need qualify McCollum's doctrine regarding these foods only so far as to specify that the leaves be not those of the goosefoot family (Chenopodiaceae) whose calcium is rendered unavailable by the oxalic acid present. With this proviso, both the milk and the green leaves will enrich the dietary both in its calcium content and in its vitamin A value; and we are finding from researches analogous to those with calcium, which have been summarized here, that health is benefited

by food of extra vitamin A value through probably at least as wide a zone as with calcium.

Moreover, extra milk brings an importantly increased intake of riboflavin, and extra green vegetables bring extra vitamin C (ascorbic acid); and of both these nutrients we also have good evidence that increasing consumption brings increasing benefit, up to levels decidedly higher than those of minimal adequacy.

Thus, in practice, there is no danger of extravagance in a policy of emphasis upon liberal dietary intake of calcium. Rather, there is the best of scientific assurance that increased prominence of milk and of well chosen green-leaf foods means an increasingly wise use of food money by the consumer, and also means increased efficiency in the farmer's use of his resources for food production.

In any country where, as in the United States, there is a considerable feeding of grain to food-producing animals, there should be a national food-and-nutrition policy, understood alike by producers and consumers of food, as to how the different classes of livestock serve the human family in this aspect of the problem of the best utilization of our food supply and of our resources for food production.

We have large areas of grazing lands not likely to be plowed or to be wanted for any other use than meat (and wool and hide) production within any future that we can foresee. Thus this country is ensured a relatively abundant meat supply from land for which there is no competing use. It is only the production of *additional* meat by the feeding of cultivated crops which comes into competition with milk and egg production.

In so far as beef animals, swine, and dairy cattle do compete for the grain which the farmer proposes to feed, the assignment of a somewhat larger part of this grain to increased milk production is an important advance in scientific economy of food-production resources. Thus the nation as well as the individual or family consumer gains by giving milk a larger place in the dietary. And this, while especially prominent from the viewpoint of calcium intake, improves the dietary in several other important nutritional factors at the same time.

Thus, at least under the food-supply conditions of the Western World, the adjustments of food habit through which one increases his food-calcium intake are practically sure to improve the dietary in other respects also, and to help the normal evolution of agriculture through advancing the economy and efficiency of use of our food resources. For milk production is inherently more economical of plowed-land resources than is meat production (Sherman, 1943, pages 177–180; 1946*a*, pages 585–595; 1946*b*, pages 93–95, 120–124, 150–163). And higher milk consumption is undoubtedly an important factor in our advancing standards of human nutrition.

There is now a strong consensus of opinion in agreement with Shohl (1939), who concluded his monograph on mineral metabolism with the observation that over the latest two or three generations the performances of growth, in height as well as weight, of each generation have exceeded those of the previous one; and that this increase presumably represents an increase in well-being and is a result of our better understanding and use of the science of nutrition.

Moreover, the years since 1939 have so concentrated scientific research upon problems of nutrition and food management that we now see more clearly than ever before, how often calcium is the determining factor in the nutritional well-being both of people and of their domestic animals, and how the nutritional guidance of production planning can improve our food supplies at once in their calcium, protein, and riboflavin contents and in their vitamin A and C values. Hence we now appreciate, as we could not before, how far-reaching are the benefits of such nutritional improvements in the lives of all members of the human family.

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